Sanquin
Blood Supply

Blood and Beyond

Scientific Report 2011

Scientific Report 2011

Dear reader,

It is again a great pleasure to present you the annual scientific report of Sanquin Blood Supply Foundation. Also in 2011, our research efforts have been strongly focused on improving our knowledge on blood cells and plasma proteins and their safe and effective application in the treatment of patients. Sanquin's research program covers the complete spectrum of blood and blood transfusion research and therefore encompasses not only translational, applied and clinical studies, but also fundamental projects that aim to unravel basic aspects related to the function of genes, proteins and cells. In this short preface I will mention only a few of the special events and achievements of 2011.

First, Peter Hordijk was appointed professor of Molecular Cell Biology of Cell Migration at the Faculty of Science of the University of Amsterdam (UvA). This appointment not only provides ample possibilities to further improve our mutual research program on advanced microscopy but also will increase our participation in student education and PhD training.

In July 2011 prenatal determination of the Rhesus D blood group was introduced in the National Dutch screening program for pregnant women as a result of a collaborative effort of Sanquin Diagnostics and the National Institute for Public Health and the Environment. The big benefit of this prenatal screening, which originated from research at our Department of Experimental Immunohematology, is that children, who are at risk for Rhesus antagonism, can be identified before they are born.

At the end of 2011, the new stem cell facility was completed. This GMP-proof facility provides the opportunity to prepare clinical grade cellular products for various clinical applications. Not only standard products, like hematopoietic stem cells, but also advanced therapy medicinal products can now be prepared in a controlled environment.

I hope you enjoy reading the annual scientific report of 2011.

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Introduction

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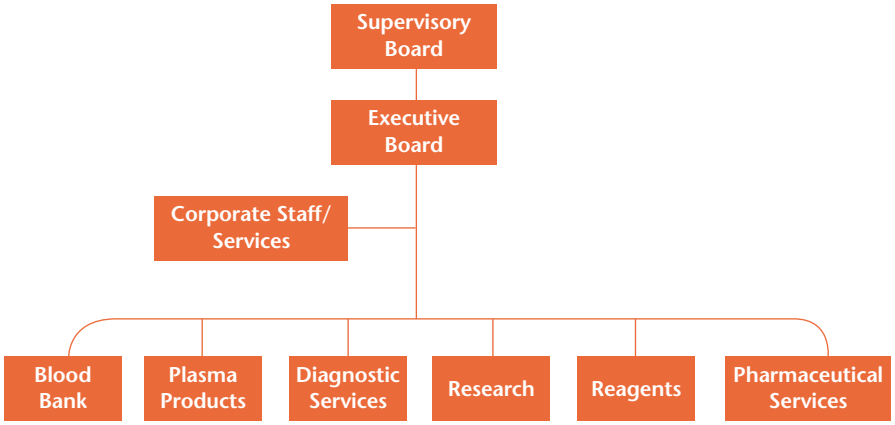
Sanquin Blood Supply Foundation

Sanquin Blood Supply Foundation comprises five divisions and a business unit. A three-member Executive Board is responsible for the organization and reports to the Supervisory Board. A corporate staff office and a number of Corporate Services support the organization. Besides the Blood Bank that operates from several locations we have the divisions Plasma Products, Diagnostic Services, Research, Reagents and the business unit Pharmaceutical Services in the Amsterdam premises. The CAF-DCF Product Development Division is located in Brussels. Besides Sanquin Research, R&D is performed in all other divisions, with an emphasis on Product and Process Development.



Research Lines

Research performed at Sanquin ranges from basic biological issues to clinical and applied issues. All research groups are headed by Principal Investigators (PI). In this scientific report you will find more information on the following research groups, ordered from basic to clinical and applied research:

Department	Research Lines	Principal Investigators
Molecular Cell Biology	Molecular Cell Biology	Prof Peter Hordijk PhD
Blood Cell Research	Phagocyte laboratory	Timo van den Berg PhD
	Laboratory for Blood Transfusion Technology	Dirk de Korte PhD
Plasma Proteins	Plasma Proteins	Prof Koen Mertens PhD
	Laboratory of Cellular Hemostasis	Jan Voorberg PhD
Hematopoiesis	Hematopoiesis	Marieke von Lindern PhD
	Laboratory of Adaptive Immunity	Martijn Nolte PhD
		Prof René van Lier MD PhD
Experimental Immunohematology	Experimental Immunohematology	Prof Ellen van der Schoot MD PhD
Immunopathology	Immunopathology	Prof Marieke van Ham PhD
	Laboratory of Autoimmune Diseases	Prof Lucien Aarden PhD
Blood-borne Infections	Blood-borne Infections	Prof Hans Zaaijer MD PhD
Transfusion Technology Assessment	Transfusion Technology Assessment	Cees van der Poel MD PhD
		Mart Jansen PhD
Transfusion Monitoring	Transfusion Monitoring	Janny de Wildt-Eggen PhD
Transfusion Medicine	Transfusion Medicine	Anske van der Bom MD PhD
		Prof Dick van Rhenen MD PhD
		Jaap Jan Zwaginga MD PhD
		Prof Anneke Brand MD PhD
Donor Studies	Donor Studies	Wim de Kort MD PhD

Scientific Advisory Board

The Scientific Advisory Board supervises the quality system, advises the Sanquin Executive Board on all matters concerning strategy, (co-ordination of) research and research infrastructure, and checks annually whether Sanquin's research program meets the framework of the policy plans. The Scientific Advisory Board also assesses the quality of Sanquin's research, based on bibliometric analyses and reports of site visits.
On 31 December 2011 the Scientific Advisory Board consisted of:

- *Prof RAW van Lier MD PhD (Chairman, Sanquin Executive Board & University of Amsterdam)*
- *Prof A Brand MD PhD (Sanquin Research & Leiden University)*
- *Prof AF Cohen MD PhD (Center for Human Drug Research & Leiden University)*
- *Prof RRP de Vries MD PhD (Leiden University)*
- *Prof DE Grobbee MD PhD (Utrecht University)*
- *Prof CE van der Schoot (Sanquin Research & University of Amsterdam)*
- *Prof DJ van Rhenen MD PhD (Sanquin Blood Bank & Erasmus University Rotterdam)*
- *JW Smeenk MSc, Executive secretary (Sanquin Research)*

Research Assessment

All Sanquin research groups are visited by an international peer review committee once every five years.
In 2011, the research group of Timo van den Berg was assessed. A number of recommendations were given and are being taken into account in 2012. As in earlier years, the Peer Review Committee was supported by an executive secretary from the independent agency Quality Assurance Netherlands Universities (QANU).

Academic affiliations

The Sanquin research departments attract many students who participate in scientific projects.
At many Dutch universities, members of the staff from various Sanquin divisions are involved in theoretical and practical training programs for undergraduate and graduate students in (medical) biology, pharmacy, medicine, and health sciences as well as for laboratory technicians. Of course, Sanquin is also involved in training specialists in blood transfusion medicine, other medical specialties, and training nurses.
Sanquin has established a recognized training program for medical doctors specializing in transfusion medicine and donor care.
Sanquin Consulting Services provides training on the job for colleagues from sister organizations in developing countries in Africa, South America, and Asia as well as the former East European countries. With the University of Groningen Medical Center, Sanquin Blood Bank runs a postgraduate masters program, under the heading of the Academic Institute for International Development of Transfusion Medicine (IDTM). Sanquin is a WHO Collaborating Organization for Transfusion Medicine.

Professorships Sanquin Staff

- *Prof Anneke Brand MD PhD (Transfusion Medicine, Leiden University Medical Center, Leiden University).*
- *Prof Peter Hordijk PhD (Molecular cell biology of cell migration, Faculty of Science, University of Amsterdam)*
- *Prof Taco Kuijpers MD PhD (Pediatric immunology, Emma Children's Hospital, University of Amsterdam)*
- *Prof Koen Mertens PhD (Pharmaceutical plasma proteins, Faculty of Pharmacy, Utrecht University)*
- *Prof Ellen van der Schoot MD PhD (Experimental immunohematology, Academic Medical Center, University of Amsterdam)*
- *Prof Marieke van Ham PhD (Biological immunology, Faculty of Science, University of Amsterdam)*
- *Prof René van Lier MD PhD (Experimental Immunology, Academic Medical Center, University of Amsterdam)*
- *Prof Dick van Rhenen MD PhD (Blood transfusion medicine, Erasmus University Medical Center, University of Rotterdam)*
- *Prof Hans Zaaijer MD PhD (Blood-borne infections, Academic Medical Center, University of Amsterdam)*

CAF-DCF professorships

- *Prof Michel Delforge MD PhD (CAF-DCF professor in Hematology and stem cell plasticity, Catholic University of Leuven)*
- *Prof Jacques Pirenne MD PhD (CAF-DCF professor in Abdominal transplant surgery, Catholic University of Leuven)*

Joint AMC – Sanquin Landsteiner Laboratory

Historically there is a strong collaboration with the Academic Medical Center (AMC) of the University of Amsterdam. The joint AMC – Sanquin Landsteiner Laboratory is housed mainly within Sanquin premises. Through this collaboration Sanquin staff members participate in AMC research programs and curricula. Researchers of Sanquin contribute to the research programs of the Center for Immunology Amsterdam (CIA) and the Center for Infection and Immunity Amsterdam (CINIMA) focusing on immunology, (immuno) hematology, and blood-borne infections.

Joint Sanquin – LUMC Jon J van Rood National Center for Clinical Transfusion Science

The Jon J van Rood National Center for Clinical Transfusion Science is a joint collaboration between Sanquin and the Leiden University Medical Center. This already long-standing collaboration is focused on translational, clinical and epidemiological studies in blood transfusion medicine. The Center is involved in training for medical specialists on blood transfusion medicine.

Within the Jon J van Rood Center, the Department of Transfusion Medicine of Sanquin Research collaborates closely with the departments of Clinical Epidemiology and Immunohematology & Blood Bank of Leiden University Medical Center. Various clinical departments of this university hospital are involved in a number of clinically relevant studies and clinical trials in blood transfusion medicine. The Cord Blood Bank is also part of the Center.

Joint Sanquin – Utrecht University Van Creveld Laboratory
Together with the Department of Pharmaceutical Sciences of Utrecht University Faculty of Science our Department of Plasma Proteins collaborates in the Van Creveld Laboratory, focusing on basic and translational research on coagulation disorders.

Joint Sanquin – Julius Center Transfusion Technology Assessment Unit
Within Julius Center for Health Sciences and Primary Care the joint Transfusion Technology Assessment Unit focuses on cost-effectiveness models and risk analyses of the blood transfusion chain. The unit is embedded in the research department of Technology Assessment. The Julius Center is part of the University Medical Center Utrecht.

Quality Assurance and Accreditation

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Code of Conduct: In 2006 the Sanquin Executive Board decided on a research Code of Conduct, based on various Codes of Conduct from Dutch Universities and the Royal Netherlands Academy of Arts and Sciences. Sanquin was awarded membership of LOWI – the national organization for scientific integrity – that acts as independent advisory body in case of a breach of scientific integrity by a Sanquin member of staff. An independent ombudsman had been appointed in 2006.
Accreditation: The Laboratory for Stem Cell Transplantation and The Cryobiology Department participated successfully in the Sanquin Multisite Certification Audit to extend their ISO 9001certification. The Laboratory for Stem Cell Transplantation also conducted its ISO 13485 certificate and JACIE accreditation.
The Blood Transfusion Technology Department was visited by the Dutch Accreditation Council (RvA) and the CCKL in March 2011. It extended its ISO 17025 accreditation and certification in accordance with the CCKL 'Code of Practice Version Four'.
Quality Management System: In 2011 a start was made on building a dedicated Quality Management System for Sanquin research. The final draft Quality Manual was presented to the management team in December 2011.
Publication: Waas JCJ. Onderzoek onderzocht, zoektocht naar een Kwaliteitsmanagement-systeem voor fundamenteel wetenschappelijk (biomedisch) onderzoek. Synaps 2011; 32:20-23

Milestones

In April 2011 the fourth two-day Sanquin Spring Seminar proved to be a success. Under the heading of "Advances in clinical transfusion science" nearly twenty speakers discussed recent results and expectations for the future of transfusion medicine.

Personnel

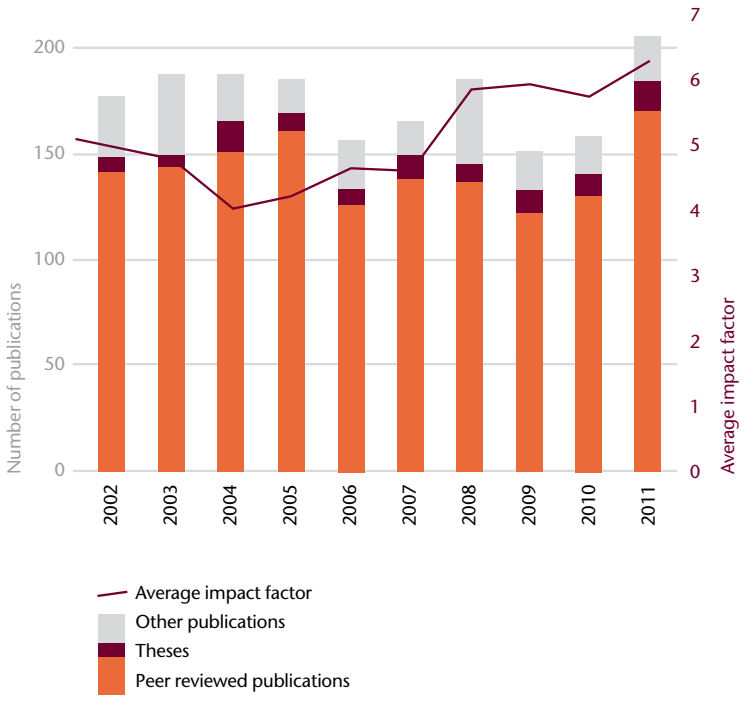
Professor Anneke Brand retired in 2011 as Professor of Transfusion Medicine at Leiden University and as head of the Research Department of Transfusion Medicine. She will continue to put her knowledge and expertise towards the international transfusion community and will be active within Europdonor.

Peter Hordijk, head of the Department of Molecular cell biology was appointed Professor of Molecular Cell Biology of Cell Migration at the University of Amsterdam. This professorship is embedded in the Swammerdam Institute of Life Sciences of the Science Faculty. Martijn Nolte started as Principal Investigator of the Adaptive Immunity research group, within the Department of Hematopoiesis.

Carlijn Voermans, manager of the Stem Cell Laboratory and researcher at the Department of Hematopoiesis, was awarded an LSBR fellowship for her application "Mesenchymal stromal cells and hematopoietic stem cell transplantation; from bench to bedside".

Scientific Publications

The number of papers in peer-reviewed journals is 170, which is comparable to previous years. The average impact factor is 6.0. The average number of citations in the five years after publishing (2006) was 23.3 for 126 papers.



Scientific papers and average impact factor

Year	Average number of citations per paper
1997	15.0
1998	20.6
1999	17.5
2000	19.7
2001	16.9
2002	21.4
2003	22.2
2004	18.3
2005	18.6
2006	23.3

Scientific publications and average impact factor
Papers* published in 1997 through 2006 annual reports cited** in five full years after publication
* Only (S)SCI AHCI papers are included
** Excluding self citations

Funding

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In 2011, Sanquin researchers were again successful in obtaining external funding, as shown in an overview of our sponsors on page 105. Several projects were applied for, ranging from European funding to Charity funds.
Fifteen research projects out of 44 were funded from Sanquin resources for product and process development for cellular products, after a review of quality by external experts and relevance to Sanquin's mission by the Research Programming Committee.
The available funds for product and process development within the organization are expected to grow slightly in the years ahead.

Valorization

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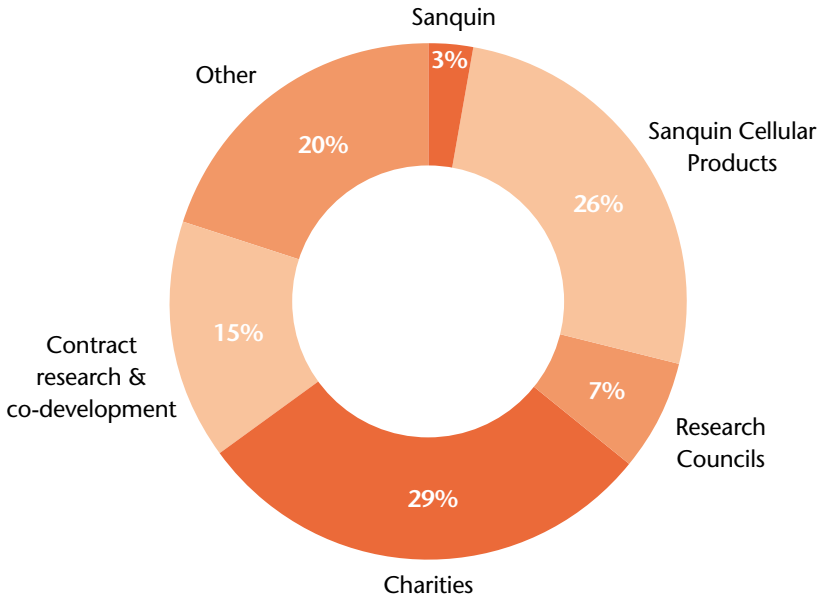
Be it in the area of therapeutic or diagnostic product development, testing of devices or process innovation, Sanquin Research is a sought-for partner in co-development and contract research activities. Within Sanquin the generated know-how and innovations are shared with our stakeholders: the Blood Bank, Plasma Products, Pharmaceutical Services, Diagnostic Services and Reagents divisions to improve patient healthcare.
Biotech, pharmaceutical, diagnostic and devices companies value Sanquin's in-depth knowledge and expertise and the translational mindset of our researchers in blood transfusion, immunology, infectious diseases, blood coagulation, hematology, hemostasis and thrombosis. Revenues generated by research collaborations, contract research and out-licensing of patents/hybridomas provide additional funding for our research activities.
An overview of commercial parties with which Sanquin Research has collaborated through the years is shown on page 105. An overview of out-licensed and available published patents and cell lines is shown on page 104.



Research project income 2011 (direct costs)

Reseo

urch
Lines

Molecular Cell Biology

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Within the department of Molecular Cell Biology we study the molecular basis of leukocyte transendothelial migration in a multidisciplinary fashion. We combine protein chemistry with cell biology and invest considerable effort in optimizing our live-cell imaging studies. We are interested in the regulation of the actin and microtubule cytoskeleton by small GTPases as this translates into the dynamics of cell adhesion and motility (Research line 1). We are also interested in the control of leukocyte-endothelium interactions by adhesion molecules such as integrins and their ligands, as well as regulatory, membrane-associate proteins such as the prion protein (Research line 2). Finally, the endothelial signaling which controls leukocyte adhesion and modulates interendothelial cell-cell contacts remains one of the central topics within the department (Research line 3).

Prof Peter Hordijk PhD, p.hordijk@sanquin.nl

Rho GTPase signaling in cell adhesion and migration

Cytoskeletal dynamics is regulated by the family of Rho-like small GTPases. We have been focusing on the Rac1 GTPase, a key member of this family that is known for its induction of actin polymerization and regulation of integrin and cadherin-based cell adhesion. In recent years we identified a series of novel Rac1-interacting proteins and have identified the biology that accompanies these interactions.

We published a study on the BAR domain protein PACSIN2, which is a novel interactor for Rac1 and which, as we discovered, is an important negative regulator of Rac1 activity, and consequently cell spreading and migration. PACSIN2 appears primarily to regulate the internalization of activated Rac1 from the plasma membrane into early endosomes. We hypothesize that this is the location where Rac1 inactivation occurs, since removal of PACSIN2, and thus less Rac1 internalization, keeps Rac1 activity high. PACSIN2 employs its BAR domain to form long inward moving tubules in areas of membrane ruffling (Figure 1), on which also Rac1 can be detected. PACSIN2 acts in conjunction with dynamin, as large GTPases that regulates membrane internalization and vesicle formation by scission.

An unusual Rac1-interacting protein that we previously identified and have worked on for several years is the ubiquitously expressed proto-oncogene SET/I2PP2A. This is a nuclear protein which does, however, exit the nucleus in a seemingly spontaneous fashion. Cytoplasmic localization of SET is promoted by Rac1 and correlates positively with cell migration. We have developed an image analysis method to quantify this nucleo-cytoplasmic shuttling behavior, which is common to many nuclear proteins. Current studies focus on finding stimuli or conditions that in fact promote the exit of SET from the nucleus, as part of a migration-stimulating signaling pathway.

Finally, we have finalized our studies on the Rac1 binding ubiquitin ligase Nedd4. This ligase does not target Rac1 itself, but we found that it ubiquitylates the adapter protein disheveled1 (Dvl1). This removal of Dvl1 appears to serve a function in controlling cell-cell contact, since loss of Nedd4 is associated with loss of cell-cell adhesion. The mechanism downstream of Dvl1 that promotes increased permeability in monolayers of epithelial and endothelial cells is currently under investigation.

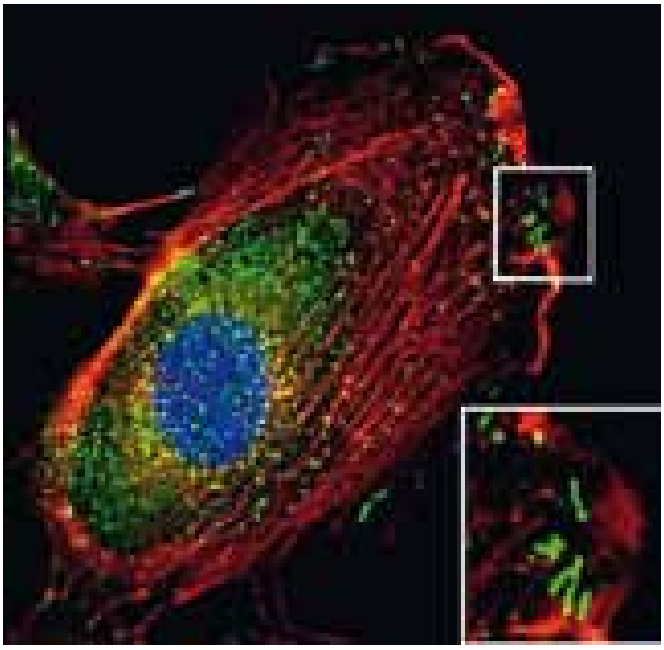

Figure 1: PACSIN2-positive tubules in primary human endothelial cells. PACSIN2 is detected in green and localizes in perinuclear vesicles as well as on short-lived tubulo-vesicular structures in the cellular periphery. F-actin is in red and the nucleus is in blue.

Key publications

De Kreuk BJ, Nethe M, Fernandez-Borja M, Anthony EC, Hensbergen PJ, Deelder AM, Plomann M, Hordijk PL. The F-BAR domain protein PACSIN2 associates with Rac1 and regulates cell spreading and migration. J Cell Sci 2011; 124:2375-88.

Nethe M, Hordijk PL. A model for phospho-caveolin-1-driven turnover of focal adhesions. Cell Adh Migr 2011; 5:59-64.

Lam BD, Anthony EC, Hordijk PL. Analysis of nucleo-cytoplasmic shuttling of the proto-oncogene SET/I2PP2A. Cytometry A 2012; 81:81-9.

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Role of the prion protein in leukocyte and endothelial cell adhesion and migration

The prion protein (PrP) is a glycoprotein expressed on the surface of most cells, in particular in nervous and immune tissues. PrP is well-known for its participation in the pathogenesis of prion diseases, also known as Transmissible Spongiform Encephalopathy. However, the physiological function of PrP remains elusive since deletion of the gene encoding for PrP in mice has no major phenotypic effects. The high degree of PrP sequence conservation in evolution (90% sequence identity between human and mouse proteins) argues in favor of an important role for PrP and the existence of other proteins that may take over the PrP function in complex organisms. In contrast, deletion of PrP in zebrafish causes an arrest in embryo development due to deficient embryonic cell-cell adhesion. We have studied the role of PrP in cell-cell and cell-extracellular matrix interactions operating during the process of leukocyte extravasation.

We have shown that PrP plays a negative role in leukocyte chemotaxis. Since the strength of cell adhesion determines the efficiency of migration, we have studied the effects of PrP down-regulation in integrin and RhoA activation. Integrins mediate cell adhesion by binding to extracellular matrix proteins while RhoA regulates de-adhesion of the trailing edge of migrating cells and it is therefore required for proper cell motility. Silencing PrP expression in leukocytes resulted in decreased integrin activation and enhanced RhoA activity. This suggests that PrP deletion has a negative effect on cell adhesion to the matrix, which explains why leukocytes are more migratory in the absence of PrP. In parallel, we have detected endogenous PrP after inducing integrin activation in leukocytes. Since leukocytes also express integrin receptors on their surface, integrin activation induces cell aggregation due to integrin/integrin receptor interactions. In these cells, endogenous PrP co-distributes with integrins at sites of cell-cell interaction (Figure 2). In addition, activation of PrP-dependent signaling also induces cell-cell aggregation. Altogether, our data suggest that PrP is a positive regulator of integrin function in leukocytes through the regulation of integrin and RhoA activation.

In support of a universal role of PrP in regulating cell adhesion, we have shown that silencing of PrP in endothelial cells also impairs adhesion to the matrix. However and in contrast to leukocytes, PrP silencing decreases endothelial cell migration. This can be explained by the absolute requirement of endothelial cells to attach to the substratum to be able to migrate.

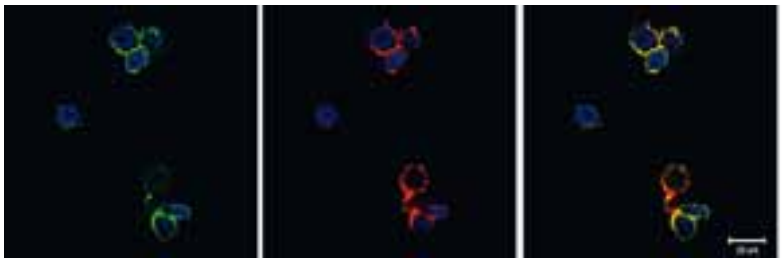

Figure 2: Human monocytic U937 cells aggregate upon integrin activation with manganese. Endogenous PrP (red) co-distributes with integrins (green) at sites of cell-cell contact.

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Role of guanine-nucleotide exchange factors in leukocyte transendothelial migration

Leukocyte extravasation describes the process by which leukocytes adhere to and transmigrate across the endothelium. Although the endothelium was originally regarded as a passive monolayer of cells, it has now become evident that it plays an active role during the extravasation of leukocytes. Our research has shown that upon integrin-mediated adhesion of leukocytes to the adhesion molecule ICAM-1 on the endothelium, the small GTPases Rac1 and RhoG are activated sequentially. Using biochemical activity assays, it became evident that the guanine nucleotide exchange factors SGEF and Trio are involved in RhoG and Rac1 activation. Depletion of SGEF or Trio expression in the endothelium or inhibiting the activity of Trio prevented primary neutrophils and monocytes from crossing the endothelial barrier under physiological flow conditions.

Upon adhesion of leukocytes, the endothelium responds by inducing large membrane protrusions, termed endothelial docking structures that surround and guide leukocytes during transendothelial migration. Detailed analysis using immunofluorescent microscopy showed that the formation of these docking structures was perturbed in Trio-silenced cells (Figure 3). Interestingly, reduction of Rac1 levels showed a similar phenotype, i.e. no docking structure formation, whereas RhoG silencing did not prevent the initial formation of the docking structures but failed to form full functional docking structures.

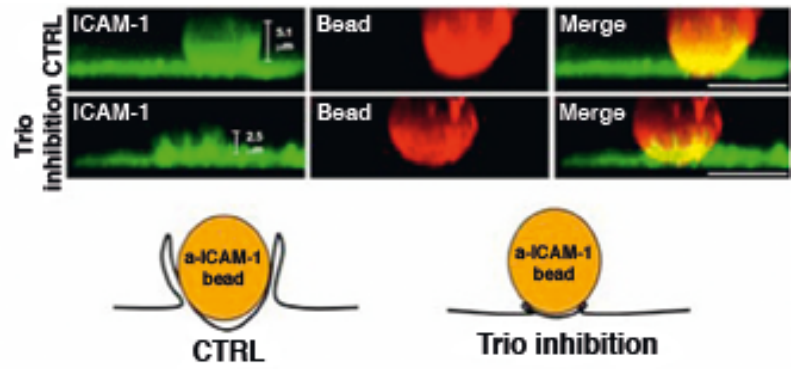

Figure 3: Anti-ICAM1 antibody-coated beads (in red) are used to cluster ICAM-1 (in green) and induce docking structures. Inhibiting Trio reduces the formation of docking structures.

In a related study, we showed that Trio regulates cell spreading and membrane dynamics. Trio can catalyze nucleotide exchange on several small GTPases, including Rac1, RhoG and RhoA. The N-terminal DH-PH domain is known to activate Rac1 and RhoG, whereas the C-terminal DH-PH domain can activate RhoA. We found that the N-terminal DH-PH domain activates Rac1 and RhoG independently from each other. In addition, we showed that the flanking SH3 domain binds to the proline-rich region of the C-terminus of Rac1, but not of RhoG. Rescue experiments in Trio shRNA-expressing cells showed that the N-terminal DH-PH domain of Trio, but not the C-terminal DH-PH domain, restored fibronectin-mediated cell spreading and migration defects that are observed in Trio-silenced cells. Kymograph analysis revealed that the N-terminal DH-PH domain, independent of its SH3 domain, controls lamellipodia dynamics. Using siRNA against Rac1 or RhoG, we found that Trio-D1-induced lamellipodia formation required Rac1 but not RhoG. Together, we conclude that the GEF Trio is responsible for lamellipodia formation through its N-terminal DH-PH domain in a Rac1-dependent manner during fibronectin-mediated spreading and migration.

Key publications

Van Rijssel J, Hoogenboezem M, Wester L, Hordijk PL, van Buul JD. The N-Terminal DH-PH Domain of Trio Induces Cell Spreading and Migration by Regulating Lamellipodia Dynamics in a Rac1-Dependent Fashion. PLoS One 2012; 7:e29912.

Krijnen PA, Hahn NE, Kholová I, Baylan U, Sipkens JA, van Alphen FP, Vonk AB, Simsek S, Meischl C, Schalkwijk CG, van Buul JD, van Hinsbergh VW, Niessen HW. Loss of DPP4 activity is related to a prothrombogenic status of endothelial cells: implications for the coronary microvasculature of myocardial infarction patients. Basic Res Cardiol 2012; 107:1-13.

Molecular Cell Biology

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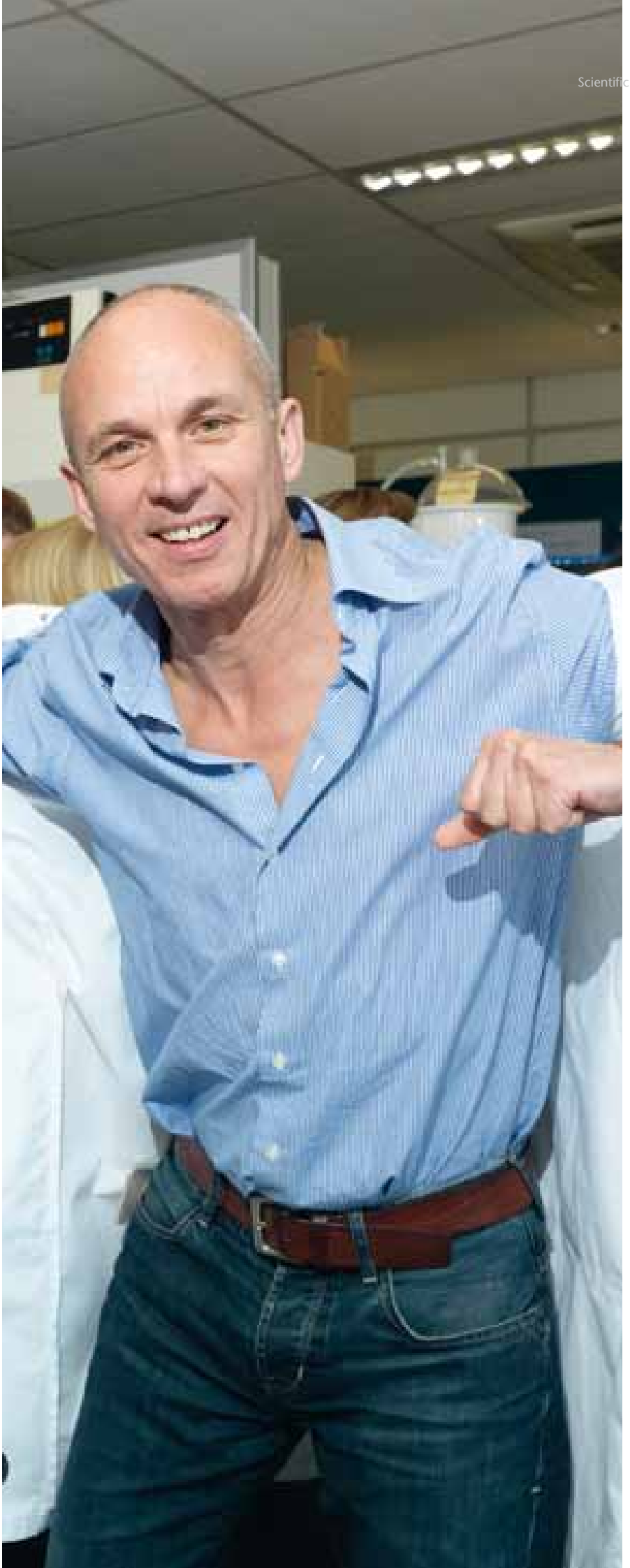

Phagocyte Laboratory

Department of Blood Cell Research

Principal Investigator:
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The department of Blood Cell Research investigates the fundamental and clinical aspects of the major human blood cell types, such as erythrocytes, platelets and phagocytes. Erythrocytes and platelets play an essential role in oxygen transport and blood clotting respectively, and both cell types are used regularly as transfusion products in patients. Phagocytes, including granulocytes, monocytes and macrophages, are critical in the host defense against infection and in the pathogenesis of various other inflammatory conditions. Phagocytes can also play a role in antibody therapy against cancer.
The aim of our research is to provide a molecular understanding of the various functions of these cells. This will allow us to maintain and further improve the quality of these cells after donation, which represents the primary focus of the Laboratory for Blood Transfusion Technology.
Our laboratory also hosts a specialized diagnostic service for phagocyte function and genetics. We are also an international center of expertise for phagocyte primary immune defects.

Research lines:
- Phagocytes
- Erythrocytes
- Platelets

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Timo van den Berg PhD, t.k.vandenberg@sanquin.nl

Phagocytes

Neutrophils and macrophages recognize pathogens by means of a variety of surface receptors. These include non-opsonic pattern recognition receptors (PRR) as well as opsonic Fc-receptors and complement receptors. Among the various classes of PRR are the leucin-rich repeat-containing families of Toll-like receptors (TLR) and NOD-like receptors (NLR), as well as C-type lectin-like receptors.

CARD9 deficiency

We identified a patient with a CARD9 deficiency, allowing us to define the role of this protein in human phagocytes for the first time. CARD9 is expressed in myeloid cells and is known to function downstream of the C-type lectin β-glucan receptors dectin-1 and dectin-2, which play a critical role in the host defense against fungi. Strikingly, the patient suffers from a rare invasive Candida infection in the brain. Our further analyses indicate a prominent role of CARD9 in Candida-induced cytokine production by monocytes and in the induction of Th17 responses. Of interest, CARD9 also appears to be essential for the killing of Candida by neutrophils.

Genetic profiling of immune receptor families

In addition to the pathways relevant for pathogen recognition and immune cell activation, we are studying the function and genetics of various families of immune receptors expressed on phagocytes and other innate immune cells. These include Fc-receptors (FcR), signal regulatory proteins (SIRP) expressed by phagocytes and killer immunoglobulin-like receptors (KIR) on natural killer cells.
The various indicated immune receptor families are subject to an extraordinary genetic variation within the human population. However, whether these individual differences, which include both polymorphisms and copy number variation (CNV), are critical determinants of individual immunogenicity and disease susceptibility has remained unknown due to the lack of appropriate technologies to address this in an integrated fashion. We are developing methodologies to be able to investigate this by employing multi-ligation probe amplification (MLPA) and large scale sequencing technology, and this year we have succeeded in doing this for the KIR, which represents the most diverse and extensive family of immune receptors.

Improving antibody therapy in cancer by targeting CD47-SIRPα interactions

SIRPα, the prototypic member of the SIRP family, is a typical inhibitory immune receptor expressed primarily on myeloid and neuronal cells. It acts as a receptor for the broadly expressed 'self' molecule CD47, and the ligation of SIRPα by CD47 results in the recruitment and activation of tyrosine phosphatases, such as SHP-1 and SHP-2, to immune receptor tyrosine-based inhibitory motifs (ITIMs) in the cytoplasmic tail of SIRPα. As a result CD47-SIRPα interactions appear to exert 'homeostatic' control over a variety of phagocyte effector functions. In addition, we also obtained evidence that interactions between CD47 on tumor cells and SIRPα on neutrophils form a critical limitation for antibody-dependent cellular cytotoxicity (ADCC) of phagocytes towards tumor cells. This is based on both *in vivo* experiments using SIRPα-mutant mice, as well as on *in vitro* evidence from ADCC experiments using a variety of human tumors and therapeutically relevant antibodies (e.g. Trastuzumab and Rituximab, see Figure 1). We have also generated novel antibodies against SIRPα that have the capacity to improve ADCC and these may be instrumental for enhancing the efficacy of antibody therapy in cancer patients.

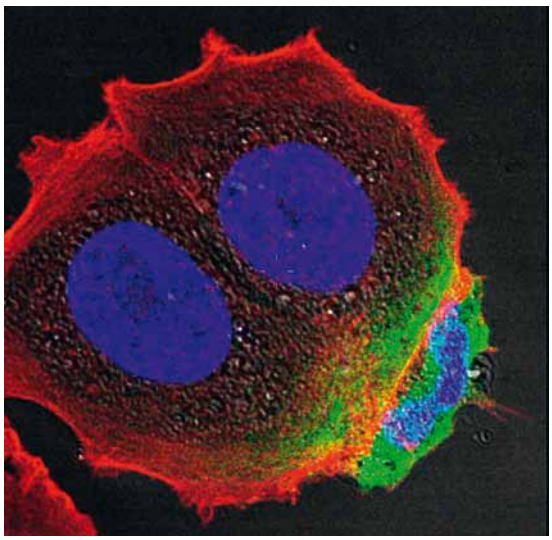

Figure 1: Immunological synapse formation during the killing of Her2/Neu-positive breast cancer cells by neutrophils in the presence of trastuzumab. Confocal microscopic image: nuclei (blue), F-actin (red), neutrophil elastase (green).

Key publications:

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Erythrocytes

This research line is focused on the function, aging and clearance of red blood cells. Red blood cells undergo changes during storage that alter their clearance and function after transfusion and there is increasing evidence that these changes contribute to the complications observed in transfused patients.

Potassium leakage during storage

Our recent findings indicate that stored erythrocytes, which are subjected to transfusion conditions *in vitro* show increased potassium leakage, hemolysis, phosphatidylserine (PS) exposure and vesicle formation. All of these effects increase with increasing storage time. Furthermore, evidence was acquired that erythrocytes can reverse PS exposure by shedding parts of their membrane as vesicles. These vesicles can serve as a platform for the coagulation cascade. Long-stored erythrocytes were shown to have decreased flippase activity and increased scrambling activity in our transfusion model, which leads to PS exposure and the release of vesicles. Lastly, potassium leakage was identified to be the cause of the decreased flippase activity. These findings reveal that potassium leakage, a well-known phenomenon of prolonged erythrocyte storage, primes the erythrocytes for PS exposure. The PS exposure will lead to vesicle formation and might have an important impact on the post-transfusion function and side-effects of stored erythrocytes.

CD47 as a molecular switch for erythrocyte clearance

We also investigated the role of CD47 in red blood cell clearance. CD47 on erythrocytes inhibits phagocytosis through interaction with the inhibitory immune receptor signal regulatory proteins (SIRP) α expressed by macrophages. Thus, the CD47-SIRPα interaction constitutes a negative signal for erythrocyte phagocytosis. However, CD47 was found not to function solely as a "don't eat me" signal for uptake, but can also act as an "eat me" signal. In particular, a subset of old erythrocytes present in whole blood was shown to bind and to be phagocytosed via CD47-SIRPα interactions. Furthermore, experimental aging of erythrocytes was found to induce a conformational change in CD47 that switches the molecule from an inhibitory signal into an activating one. Pre-incubation of experimentally-aged erythrocytes with human serum prior to the binding assay was required for this activation. Aged erythrocytes were found to have the capacity to bind the CD47-binding partner thrombospondin-1 (TSP-1) and treatment of aged erythrocytes with a TSP-1-derived peptide enabled their phagocytosis by human red pulp macrophages. Finally, CD47 on erythrocytes that had been stored for a prolonged time was shown to undergo a conformational change and bind TSP-1. These findings reveal a more complex role for CD47-SIRPα interactions in erythrocyte phagocytosis, with CD47 acting as a molecular switch for controlling erythrocyte phagocytosis.

Key publications:

Burger P, Hilarius-Stokman P, de Korte D, van den Berg TK, van Bruggen R. CD47 functions as a molecular switch for erythrocyte phagocytosis. Blood 2012 March 16 [Epub ahead of print]

Burger P, Korsten H, Verhoeven AJ, de Korte D, van Bruggen R. Collection and storage of red blood cells with anticoagulant and additive solution with a physiologic pH. Transfusion 2012 Jan 10 [Epub ahead of print]

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Platelets

Platelets play a central role in coagulation and in this line the molecular mechanisms of platelet formation and function are being studied.

Consolidation of the flow cytometry-based platelet aggregation assay (FCA)

We have developed a flow-cytometry based platelet aggregation assay (FCA) and in the past year we have further optimized it for use in animal models such as mice. We have developed a whole panel of agonists and antagonists that can be used to measure platelet aggregation in washed platelets or whole blood from adult mice but also from embryonic blood. In addition, we have implemented FCA to assess the influence of blood group and plasma on platelet function (ongoing study), and the effect of anti-pathogen treatments of platelet concentrates (i.e. Mirasol PRT) on platelet function before and after transfusion (sharing patient cohort with the PREPAReS/PATS study, de Korte D, van der Meer P, Middelburg RA and Zwaginga JJ). It is our aim to study whether FCA could be used as a transfusion prognostic test when analyzing the function of transfused-to-be platelets with the recipient's plasma.

Caprin2 in megakaryopoiesis

In our quest to find novel regulators of the megakaryopoietic process, we focused on Caprin2, which was initially described as germ cell tumor 1 (GCT1), a novel gene overexpressed in human testicular seminomas, located in chromosome 12 (12p11), a hot spot for chromosomal aberrations linked to carcinogenic manifestations in the testis. Soon after, it was reported that Caprin2 is expressed in erythroid cells and upregulated upon erythroid differentiation. Remarkably, its over-expression leads to a decrease in cell growth rate and apoptosis. Caprin2 protein has a C1q domain, and it can heterotrimerize and locate to the cell surface displaying a tumor necrosis factor-like domain. Recently, there have been two publications linking Caprin2 with canonical Wnt signaling in zebra fish and with FGF signaling in the mouse lens; however, the molecular mechanism is not yet understood. In addition, Caprin2 has two RNA-binding domains, and locates in RNA granules in neurons.
To study the role of Caprin2 in human megakaryopoiesis, we have used DAMI megakaryoblastic leukemia cell line and lentiviral-mediated short-hairpin RNA (shRNA) technology (Figure 2). Knock-down of Caprin2 resulted in decreased cell proliferation, higher ploidy status and decreased expression levels of several megakaryocytic surface markers (cKit, CD41, CD61 and CD63). Levels of GATA1, as well as its target gene cMPL/thrombopoietin receptor, were increased and expression of the antagonizing transcription factor PU.1 was decreased. Preliminary results indicate that phosphorylated retinoblastoma protein (pRB) sequestered by excess GATA1 could be the reason for cell cycle defects and aberrant ploidy status. In addition, the number of $CD61^{+}/CD41^{+}$ platelet-like particles that are shed by megakaryocytes were decreased in Caprin2 knock-down cells. We hypothesize that Caprin2 regulates translation of Gata1 mRNA, and we are currently characterizing Caprin2 interacting partners and Caprin2-RNA granules specific mRNAs in megakaryocytes.

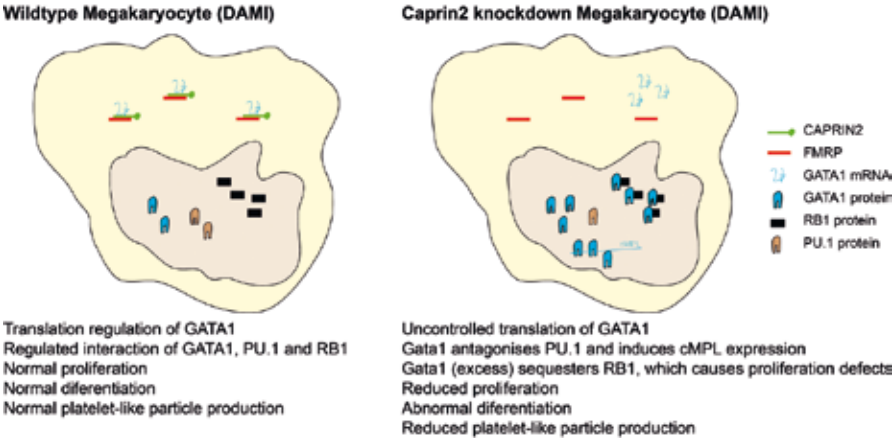

Figure 2: the effects of caprin2 deficiency in megakaryopoiesis

Key publications:

Van de Vijver E, de Cuyper IM, Gerrits AJ, Verhoeven AJ, Seeger K, Gutiérrez L, van den Berg TK, Kuijpers TW. Defects in Glanzmann thrombasthenia and LAD-III (LAD-1/v) syndrome: the role of integrin 1 and 3 in platelet adhesion to collagen. Blood 2012; 119:583-6.

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The laboratory for Blood Transfusion Technology performs applied research to increase the knowledge of current and future blood products as well as materials. New devices and materials are evaluated, and methods to evaluate the quality of blood products are developed and/or improved. Special attention is given to the link between *in vitro* and *in vivo* evaluation through cooperation with the Department of Transfusion Medicine. The Principal Investigator is also senior scientist in the phagocyte laboratory of the Department of Blood Cell Research, and is involved in more fundamental projects on erythrocytes and platelets. In this way the Blood Bank has early access to results obtained in the Research division for use in product development and improvement.

Research Lines:

- Improving materials and methods for storage of blood components
- Bacterial safety of blood products
- *In vitro* quality tests for cellular blood products

Improving materials and methods for storage of blood components

The Blood Bank is a producer of blood products under Good Manufacturing Practice conditions and needs to know the limits of the methods in use, for example with respect to temperature effects during collection and preparation. Some studies in this field were performed in 2011. Another aspect of this research line is the quality of blood components in relation to the use of alternative plasticizers for blood bag foils.

Storage of whole blood at +18°C or +25°C and the effect on blood component quality

Based on European Directives, the storage temperature for whole blood (WB) is +20°C to +24°C. In the blood bank, room temperature mostly varies between +18°C and +25°C. It was investigated whether the quality of blood components was affected by initial storage for 24 h (maximal allowed time until component preparation) at +18°C or +25°C (worst case scenario). For this study, after collection under standard conditions, two series of WB were placed in a climate cabinet for 24 h, one series at +18°C and one series at +25°C. After 24 h the WB was processed into white blood cell-reduced red blood cells (RBCs), buffy coat (BC), and plasma. The BCs were further processed into platelet concentrates (PCs) derived from a single BC. Only minimal differences were found for the WB stored under different conditions and for the various components prepared from this WB, which were subsequently stored under standard conditions. It was concluded that the current limits should be maintained, but that deviations during the first 24 h of storage within the range +18°C to +25°C would be acceptable.

Use of DEHP-free blood bags for collection and storage of blood products

The plasticizer di(2-ethylhexyl)-phthalate (DEHP) is a common component in medical plastics. DEHP is non-covalently bound to the PVC polymer and can leach from PVC devices when the surface comes into contact with fluids. There are concerns that exposure to phthalates might induce developmental and reproductive toxicity. In blood collection systems DEHP is also often used as a plasticizer. Fresenius HemoCare Netherlands BV has developed a whole blood collection system of which all components are made of non-DEHP PVC. In these systems, the tubings and bags were made from DINCH (Hexamoll diisononyl-1,2-cyclohexane dicarbonic acid, BASF Corp., Germany) plasticized PVC. The *in vitro* quality of plasma and red blood cell concentrates, collected and stored in a DINCH system was compared to products in the conventional DEHP-containing system. Whole blood (500 ml ± 10%) was collected into DEHP-PVC (n=37) and DINCH-PVC (n=38) collection systems. After overnight hold, WB was centrifuged and separated into plasma, buffy coat and RBCs. Plasma was assayed for coagulation and activation markers. After the addition of additive solution, SAG-M or PAGGS-M, the RBCs were leukodepleted and stored at 2-6°C for 42 days. A panel of *in vitro* red cell characteristics was determined during storage. DEHP and DINCH levels were determined at the beginning and end of the storage period.

The complete absence of DEHP in the collection system has no effect on WB processing and plasma coagulation characteristics. During storage of RBCs in SAGM, the absence of DEHP resulted in increased cell swelling and hemolysis, but all other parameters showed similar changes. With alternative additive solutions, like PAGGSM, the absence of DEHP had much less detrimental effects on cell swelling and red cell stability. Leakage of DINCH into the blood product during storage was much less pronounced than that of DEHP.

Key publications

Sampson J, de Korte, D. DEHP-plasticized PVC: relevance to blood services. Transfus Med 2011; 21:73-83.

Bontekoe IJ, van der Meer PF, de Korte, D. Effect of rate and delay of cooling during initial cooling process: in vitro effect on red cells. Vox Sang 2011; 101:16-20.

Bacterial safety of blood products

Contamination of platelets with bacteria is a major microbiological risk in blood transfusion. This applies especially to platelet concentrates (PCs) because their storage conditions, at room temperature and under constant agitation, support bacterial growth. Therefore both the prevention of contamination and gathering knowledge on the type of contamination are the subject of research within the blood bank. 2011 marked 10 years since screening of PCs was started in the Netherlands.

10 years of screening for bacterial contamination of platelet concentrates in the Netherlands

As contamination of platelets with bacteria is a major microbiological risk in blood transfusion, screening for bacterial contamination can reduce the frequency of bacterial transmission considerably. Since 2001, all PCs produced in the Netherlands are cultured with the BacT/Alert culturing system with large volume (7.5 ml) cultures in both an aerobic and anaerobic bottle. About 63,000 pooled buffy coat derived PCs are produced per year and about 4000 aphaeresis PCs are collected. PCs are released on a 'negative to date' basis. When a PC from pooled buffy coats is flagged positive for bacterial growth, the 5 related red blood cell (RBC) concentrates are also cultured for bacterial growth. Due to the short shelf life it was decided to perform no retesting on PCs, but to destroy the product. However, RBCs are released again if negative during the 7-days culture.

Due to the principle of 'negative to date', PCs which are already transfused can have a positive culture result afterwards. If this is the case, the hospital is contacted and it is explained that there is a chance that the transfused unit was bacterially contaminated. It is also explained that the growth in the culture is normally ahead of the growth in the PC from which the sample was taken and that a bacterial transmission by the PC would be rare. This message was very well understood and accepted by the clinicians. During the years in which 100% screening was applied, the number of units released as 'negative to date' with a positive culture after being transfused, decreased substantial by introduction of the deviation bag. In the initial years we had about 230 units a year being released as 'negative to date', but with a subsequent positive culture after transfusion. After introduction of the deviation bag this decreased to a mean of 110 per year. Over a period of 4 years (2006-2009) an active look-back was undertaken for 435 patient records of these transfusions and only three cases with a reported transfusion reaction were found. For these cases in which a transfusion reaction was reported the imputability of being related to the transfusion of a contaminated PC was unlikely.

The diversion of the first volume of collected blood was introduced by Sanquin in 2004. As a result the number of positive screening cultures decreased significantly from 0.85% to 0.37%. The number of transfusion-transmitted bacterial infections by PCs is currently less than 1 per 2 years in the Netherlands. There are no indications that 'false negative' cultures add a high risk to the transfusion of PCs. Looking back over 10 years of bacterial screening system for PCs, the conclusion was that the system as applied in Sanquin, in combination with diversion of the first collected blood, resulted in a safe system with respect to microbiological infection due to platelet transfusions.

Key publications

De Korte, D. 10 Years' Experience with Bacterial Screening of Platelet Concentrates in the Netherlands. Transfus Med Hemother 2011; 38(4):251-4.

Rood IG, de Korte D, Savelkoul PH, Pettersson A. Molecular relatedness of Propionibacterium species isolated from blood products and on the skin of blood donors. Transfusion 2011; 51(10):2118-24.

In vitro quality tests for cellular blood products

Although the final proof for the quality of cellular blood products is still the result after clinical use, *in vitro* tests can be of value to predict in vivo behavior. In 2011 a relatively new test was added to the *in vitro* set of parameters for platelets, the ThromboElastoGraphy or TEG. In 2011 the effect of lipemic plasma on blood products was also investigated with *in vitro* parameters.

Use of ThromboElastoGraphy in product evaluation
Thromboelastography (TEG) monitors the thrombodynamic properties of blood as it is induced to clot under a low-shear environment resembling sluggish venous flow. The patterns of change in shear-elasticity enable the determination of the kinetics of clot formation and growth as well as the strength and stability of the formed clot. The strength and stability of the clot provide information about the ability of the clot to perform the work of haemostasis, while the kinetics determines the adequacy of quantitative factors available to clot formation. With TEG the quantitative clot formation and the kinetics are measured, followed by measurement of the clot strength and stability, plus the clot resolution (fibrinolysis).
In the citrate-kaolin (CK) test, the intrinsic pathway is activated with kaoline (after addition of calcium, because citrate anti-coagulated blood is used) and a clot is formed by fibrinogen and platelets. In the citrate-functional-fibrinogen (CFF) test the extrinsic pathway is activated by tissue factor addition (also after calcium addition), whereas the platelet aggregation is inhibited, therefore the strength of the formed clot is representative for the amount of functional fibrinogen.
To obtain information on the normal values and the extent of variation in testing with the TEG due to donor variation, whole blood from 100 different donors was tested with the CK and the CFF test. In total the tests from 95 donors were evaluable (tested between 30 and 120 min after collection, mean 65 ± 29 min). For both tests normal values were defined for the various measured parameters (mean with 95% CI). The moment of testing for citrate blood is of importance as well as the concentration of citrate. There are also some differences between men and women. In general, women have a more rapid clot formation and the strength of the clot is higher. Whereas the concentration of fibrinogen showed a clear correlation with the maximal clot strength in the CFF results, the concentration of platelets did not show a correlation with the clot strength. This might be due to the fact that very low and very high platelet counts were not represented in the tested whole blood samples from healthy voluntary blood donors.
The project will have a follow-up in 2012 with measurement of platelet concentrates from different aphaeresis donors, to investigate donor variation in the product and to check whether TEG analyses on either product or whole blood can be used to select donors.

Effect of lipemic plasma on the *in vitro* quality of erythrocytes or platelets during storage

Dutch guidelines for blood transfusion require that plasma should not be turbid or lipemic (not milky). However, no data on the effect of lipemic plasma on the quality of cellular blood components during storage are available. Therefore the effect of lipemic plasma on the quality of cellular blood components during storage was investigated.
Either whole blood units that were discarded from regular component production because of lipemic plasma, or released units, were used for further processing. Whole blood (500 ± 50 ml) was collected in citrate phosphate dextrose, centrifuged and separated automatically with the Compomat™ G5 into a plasma, buffy coat (BC) and red blood cell (RBC) concentrate. Plasma was sampled for triglyceride analysis. After the addition of SAGM, the RBC concentrates were leukodepleted by filtration. RBC concentrates were stored at 2-6°C for 42 days and sampled at regular intervals for *in vitro* analysis. BCs were used to make a single donor platelet concentrate (SD-PC) in plasma. SD-BCs were stored on a flatbed shaker at 20-24°C and sampled at days 1, 6 and 8 for *in vitro* analysis.
Cellular components made from lipemic blood (n=8) were compared with those made from regular, non-lipemic blood (n=11).
The triglyceride concentration of lipemic plasma and normal plasma was 6.9 ± 3.0 and 1.5 ± 0.6 mmol/L respectively. The results of SD-PC during storage are shown in Table 1. Platelets stored in lipemic plasma showed a stronger decline in pH and swirling as compared to storage in regular plasma. Metabolic activity, as measured by lactate production, and activation (number of CD62 positive cells) were more pronounced during storage in lipemic plasma. The high numbers of Annexin V-positive cells combined with the high oxygen tension and the decline in platelet count, suggest that a vast majority of platelets is apoptotic after 8 days' storage in lipemic plasma.
Red cells prepared from lipemic whole blood showed significantly higher levels of hemolysis during storage. The level of hemolysis correlated with the triglyceride concentration in the plasma: in particular, RBC concentrates made from WB with a triglyceride concentration above 10 mmol/L showed high levels of hemolysis. For the other *in vitro* parameters, including glucose and ATP levels, no significant difference between lipemic RBCs and regular RBCs could be observed.
It can be concluded that lipemic plasma has a negative effect on the *in vitro* quality of both platelets and red cells during storage, and discard of the whole unit in case of lipemic plasma seems valid. Further research is necessary to determine the mechanism for the effect of lipids on cellular blood products and the maximal acceptable degree of lipemia.

	Normal plasma (n=11)		Lipemic plasma (n=8)	
SD-PC	Day 1	Day 8	Day 1	Day 8
Plt (x10^9)	69 ± 16	63 ± 12	64 ± 14	46 ± 11#
Swirl	3.0 ± 0.2	2.3 ± 0.9	2.3 ± 0.5#	0.1 ± 0.2#
pH (at 37°C)	6.99 ± 0.01	6.84 ± 0.31	7.08 ± 0.03	6.41 ± 0.47#
pO2 (mm Hg)	106 ± 17.6	64 ± 17.1	131 ± 5.8#	121 ± 31.0#
Lactate (mmol/L)	7.3 ± 0.8	17.9 ± 7.1	5.5 ± 0.8#	26.4 ± 7.8
CD62P pos. cells (%)	6.6 ±1.9	39.7 ± 16.6	18.6 ± 6.9#	39.6 ± 18.4
Annexin V pos. cells (%)	3.7 ± 1.6	36.1 ± 8.7	3.4 ± 3.4	67.6 ± 15.7#
RBC conc.	Day 1	Day 42	Day 1	Day 42
Hemolysis (%)	0.05 ± 0.02	0.35 ± 0.08	0.07 ± 0.08	0.58 ± 0.41#
Glucose (mmol/L)	31.6 ± 1.6	18.4 ± 1.7	31.1 ± 1.1	16.9 ± 2.1
ATP (µmol/g Hb)	5.69 ± 0.34	3.08 ± 0.34	5.42 ± 2.69	2.69 ± 0.56

Table 1: In vitro measures of SD-PCs and RBC concentrates during storage. Values are expressed as mean ± SD. #: p value <0.05 as compared to normal plasma (t-test).

Key publications

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Van der Meer PF, Cancelas JA, Vassallo RR, Rugg N, Einarson M, Hess JR. Evaluation of the overnight hold of whole blood at room temperature, before component processing: platelets (PLTs) from PLT-rich plasma. Transfusion 2011; 51 Suppl 1:45S-49S.

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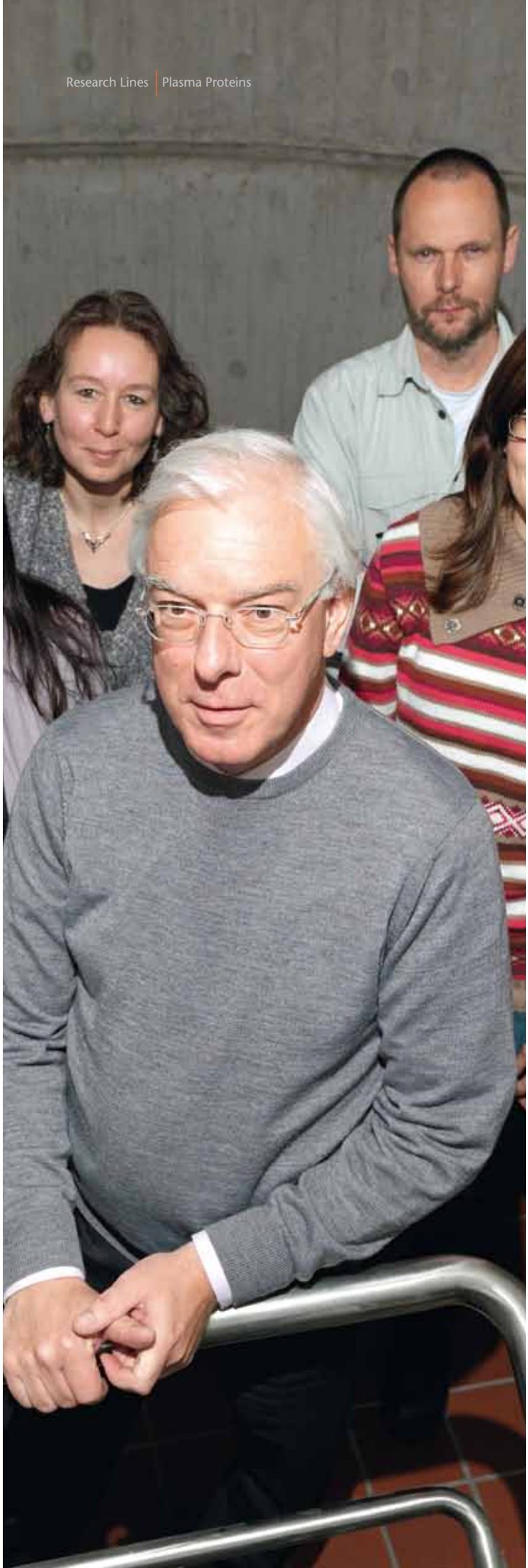

Plasma Proteins

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Research at the Department of Plasma Proteins is performed by two Principal Investigators, Prof Koen Mertens PhD and Jan Voorberg PhD. Both PI's are mutually involved within the overall focus on Hemostasis and Thrombosis on the different research lines. The research lines of Koen Mertens are described here, while research lines by Jan Voorberg also involving Koen Mertens can be found with Cellular Hemostasis (page 36).

Research lines:

- Structure and function of coagulation factors
- Cellular receptors involved in the uptake of coagulation factors
- Proteomics and biomolecular mass spectrometry of hemostatic processes

Structure and function of coagulation factors

The coagulation cascade comprises several serine proteases that act in combination with a non-enzymatic co-factor on phospholipid-containing membranes. Over the past decade we have been focusing on the mechanism by which activated factor IX assembles with its co-factor, factor VIII. These proteins are indispensable for proper functioning of the coagulation cascade as a functional absence of factor VIII (FVIII) and factor IX is associated with the bleeding disorders hemophilia A and hemophilia B. FVIII is composed of a series of repeated domains which appear in the order A1-a1-A2-a2-B-a3-A3-C1-C2. The A-domains of FVIII mediate the binding to activated factor IX and factor X, whereas the C2 domain has been implicated in binding to phospholipids. After activation, the A2 domain rapidly dissociates from activated factor VIII (FVIIIa) resulting in a dampening of the activity of the activated factor X-generating complex. The amino acid residues that affect A2 domain dissociation are therefore critical for the FVIII co-factor function. We have now employed chemical footprinting in conjunction with mass spectrometry to identify lysine residues that contribute to the stability of activated FVIII. We hypothesized that lysine residues, which are buried in FVIII and surface-exposed in dissociated activated FVIII (dis-FVIIIa), may contribute to interdomain interactions (Figure 1). Mass spectrometry analysis revealed that residues K1967 and K1968 of region T1964-Y1971 are buried in FVIII and are exposed to the surface in dis-FVIIIa. This result, combined with the observation that the FVIII variant K1967I is associated with hemophilia A, suggests that these residues contribute to the stability of activated FVIII. Kinetic analysis revealed that the FVIII variants K1967A and K1967I exhibit an almost normal co-factor activity. However, these variants also showed an increased loss in co-factor activity over time compared with that of FVIII wild type (WT). Remarkably, the co-factor activity of a K1968A variant was enhanced and sustained for a prolonged time relative to that of FVIII WT. Surface plasmon resonance analysis demonstrated that A2 domain dissociation from activated FVIII was reduced for K1968A and enhanced for K1967A. In conclusion, mass spectrometry analysis combined with site-directed mutagenesis studies revealed that the lysine couple K1967-K1968 within region T1964-Y1971 has an opposite contribution to the stability of FVIIIa.

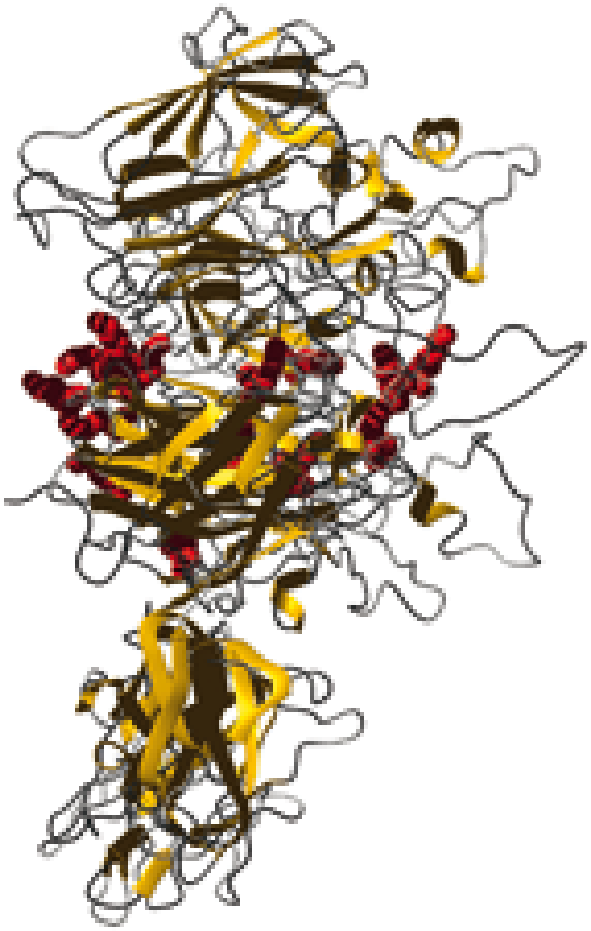

Figure 1: Surface-exposed residues (in red) involved in retaining the A2 domain in activated FVIII

Key publication

Bloem E, Meems H, van den Biggelaar M, van der Zwaan C, Mertens K, Meijer AB. Mass spectrometry-assisted study reveals that lysine residues 1967 and 1968 have opposite contribution to stability of activated Factor VIII. J Biol Chem 2012; 287(8):5775-83.

Cellular receptors involved in the uptake of coagulation factors

LDL receptor-related protein (LRP) contributes to the clearance of coagulation factor VIII (FVIII) from the circulation. Ligand binding of the low-density lipoprotein (LDL) receptor family is mediated by clusters of small complement-type repeats (CR). It has been proposed that at least two CRs are required for high-affinity interaction by utilizing two spatially distinct lysine residues on the ligand surface. LRP mediates the cellular uptake of a multitude of ligands, some of which bind LRP with a relatively low affinity suggesting a suboptimal positioning of the two critical lysines. We have recently addressed the role of the two critical lysines not only for LRP binding but also for endocytosis, initially by employing Receptor Associated Protein (RAP) as a model ligand. Variants of the third domain (D3) of RAP were constructed in which lysines were replaced by alanine or arginine at the putative contact residues K253, K256 and K270. Surface Plasmon Resonance revealed that replacement of K253 has no effect on high-affinity LRP binding at all whereas replacement of either K256 or K270 markedly reduced the binding affinity. The interaction was completely abolished when both lysines were replaced. Substitution by either alanine or arginine exerted an almost identical effect on LRP binding, suggesting arginine residues do not support receptor binding. Confocal microscopy and flow cytometry studies revealed surprisingly that the single mutants were still internalized by cells. We therefore propose that the presence of only one critical lysine is sufficient to drive endocytosis.

We next set out to identify the contribution of lysines in the interaction between FVIII and LRP. We have established that antibody fragment KM33 inhibits the co-factor function of FVIII by interacting with the membrane binding region K2092-F2093 of the C1 domain. As KM33 also blocks the interaction between LRP and FVIII, we now assessed the role of K2092 for LRP-dependent endocytosis. For this purpose, we employed FVIII-YFP derivatives and U87MG cells which express high levels of LRP. Confocal microscopy studies and flow cytometry analysis combined with siRNA technology showed that the fluorescent FVIII derivatives are indeed internalized effectively by U87MG cells in a LRP-dependent manner. Competition experiments employing an antagonist of the LDL receptor family members revealed that there is a cell surface binding event for FVIII, which is independent of LRP. Cell surface binding proved to be less effective for the FVIII-YFP variants K2092A, F2093A and K2092A/F2093A. Surface plasmon resonance analysis showed that these substitutions affect LRP binding as well. Finally, flow cytometry analysis revealed a major reduction of endocytic uptake of these FVIII-YFP variants. Our results demonstrate that C1 domain residues K2092-F2093 are of major importance for FVIII endocytosis by contributing to cell surface binding and receptor binding.

Key publications:

Van den Biggelaar M, Sellink E, Klein Gebbinck JW, Mertens K, Meijer AB. A single lysine of the two-lysine recognition motif of the D3 domain of receptor-associated protein is sufficient to mediate endocytosis by low-density lipoprotein receptor-related protein. Int J Biochem Cell Biol 2011; 43(3):431-40.

Meems H, van den Biggelaar M, Rondaij M, van der Zwaan C, Mertens K, Meijer AB. C1 domain residues Lys 2092 and Phe 2093 are of major importance for the endocytic uptake of coagulation factor VIII. Int J Biochem Cell Biol 2011; 43(8):1114-21.

Proteomics and biomolecular mass spectrometry of hemostatic processes

The binding between individual proteins involved in a hemostatic process, like blood coagulation, is typically part of a complex protein-protein interaction network. Taking maximum advantage of the versatility of the nano-LC LTQ Orbitrap XL ETD mass spectrometer, several studies have been initiated to unravel complex mechanisms involved in protein-protein interactions, storage of hemostatic proteins in the secretory organelles, and intracellular processing of proteins. An example of the last study involves immune tolerance induction against coagulation factor VIII (FVIII). Activation of T-helper cells is dependent upon the appropriate presentation of antigen-derived peptides on MHC class II molecules expressed on antigen presenting cells. In this current study we explored the repertoire of peptides presented on MHC class II molecules on human monocyte-derived dendritic cells (moDCs) from four HLA-typed healthy donors. MHC class II-bound peptides could be routinely recovered from small cultures containing 5×10^6 cells. A fraction of the identified peptides were derived from proteins localized in the plasma membrane, endosomes, and lysosomes, but the majority of peptides that were presented on MHC class II originate from other organelles. We subsequently studied the antigen-specific peptide repertoire after endocytosis of a soluble antigen. Blood coagulation FVIII was chosen as the antigen since our current knowledge of MHC class II-presented peptides derived from this immunogenic therapeutic protein is limited. Analysis of the total repertoire of MHC class II-associated peptides revealed that per individual sample, 20-50 FVIII-derived peptides were presented on FVIII-pulsed moDCs. Repertoires of FVIII-derived peptides eluted from moDCs derived from a panel of four HLA typed donors revealed that some MHC class II-presented FVIII peptides were presented by multiple donors, whereas the presentation of other FVIII peptides was donor-specific. In total, 32 different core peptides were presented on FVIII-pulsed moDCs from four HLA-typed donors. Together our findings provide an unbiased approach to identify peptides that are presented by MHC class II on antigen-loaded moDCs from individual donors.

Key publications

Van Haren SD, Herczenik E, ten Brinke A, Mertens K, Voorberg J, Meijer AB. HLA-DR-presented peptide-repertoires derived from human monocyte-derived dendritic cells pulsed with blood coagulation factor VIII. Mol Cell Proteomics 2011; 10(6):M110.002246.

Bloem E, Meems H, van den Biggelaar M, van der Zwaan C, Mertens K, Meijer AB. Mass spectrometry-assisted study reveals that lysine residues 1967 and 1968 have opposite contribution to stability of activated Factor VIII. J Biol Chem 2012; 287(8):5775-83.

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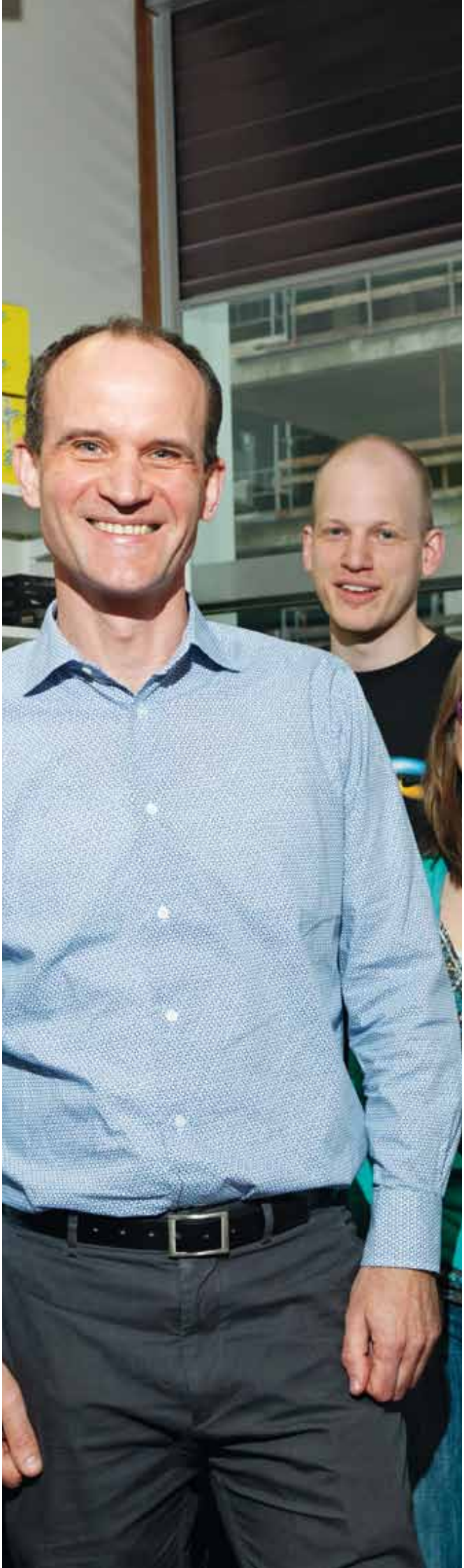
Conclusions



Laboratory of Cellular Hemostasis

Department of Plasma Proteins

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Research at the Department of Plasma Proteins is performed by two Principal Investigators, Prof Koen Mertens PhD and Jan Voorberg PhD. Both PI's are mutually involved within the overall focus on Hemostasis and Thrombosis on the different research lines.
The research lines of Jan Voorberg are described here, while research lines by Koen Mertens also involving Jan Voorberg can be found with the Department of Plasma Proteins (page 30).

Research lines:

- Biosynthesis of von Willebrand factor
- Immune response to hemostatic proteins
- Thrombotic thrombocytopenic purpura

Biosynthesis of von Willebrand factor

Biosynthesis of von Willebrand factor (VWF) occurs in vascular endothelial cells and megakaryocytes. In endothelial cells VWF is stored in rod-shaped endothelial cell-specific storage organelles, the Weibel-Palade bodies. Besides VWF, these Weibel-Palade bodies contain a number of other proteins, including P-selectin, angiopoietin-2, osteoprotegerin and a number of other components. Upon stimulation of endothelial cells by agonist such as thrombin or epinephrine, Weibel-Palade bodies undergo exocytosis, resulting in the release or surface expression of their contents. The elongated shape of Weibel-Palade bodies has been attributed to the packaging of VWF multimers into helical structures. Analysis by electron microscopy reveals tubular-like structures that most likely are composed of tightly packed helically organized VWF multimers. Following their release of Weibel-Palade bodies, VWF tubules are rapidly converted into ultra-large VWF strings that are anchored to the surface of endothelial cells. These ultra-large VWF strings provide multiple attachment sites for blood platelets (see Figure 1A+B).

During biogenesis of Weibel-Palade bodies, VWF assembles into long, slightly twisted tubules that determine the typical cigar-shaped, elongated appearance of these organelles. We have previously shown that FVIII is targeted to Weibel-Palade bodies upon its over-expression in endothelial cells. The presence of FVIII transforms the rodshaped Weibel-Palade bodies into spherical organelles. Using immuno-electron microscopy we confirmed the presence of FVIII and VWF in spherical or “potato”-like structures. Correlative light and electron microcopy revealed that FVIII-containing Weibel-Palade bodies possess a limited number of disorganized tubules that contrast sharply with the tight parallel packaging of VWF tubules in “normal” Weibel-Palade bodies. These data show that FVIII interferes with the formation of VWF tubules in newly forming Weibel-Palade bodies. These results suggest that the parallel tubular alignment of VWF tubules is crucial for the rod-shaped appearance of Weibel-Palade bodies.

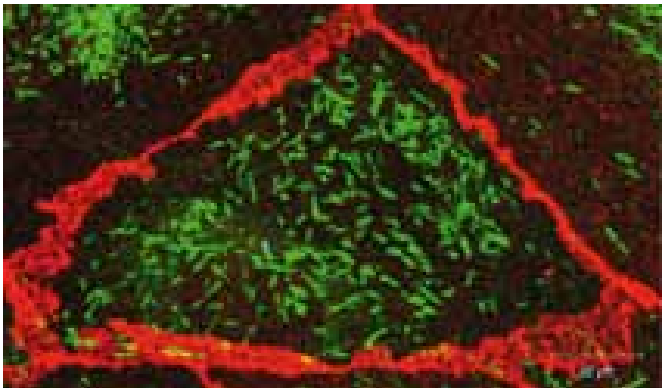

Figure 1A: Rod shaped Weibel-Palade bodies (green) in endothelial cells. The periphery of the cell is shown by staining for ß-catenin (red).

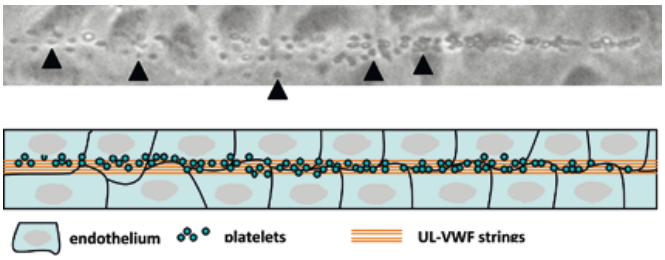

Figure 1B: Ultra-large VWF strings on the surface of endothelial cells. Stings are visualized by adhering bloodplatelets (arrowheads).

Key publications

Bouwens EA, Mourik MJ, van den Biggelaar M, Eikenboom J, Voorberg J, Valentijn KM, Mertens K. Factor VIII alters tubular organization and junctional properties of von Willebrand factor stored in Weibel Palade bodies. Blood 2011; 118:5947-56.

Valentijn KM, Sadler JE, Valentijn JA, Voorberg J, Eikenboom J. Functional architecture of Weibel-Palade bodies. Blood 2011; 117:5033-43.

Immune response to hemostatic proteins

Hemophilia

Hemophilia is an X-linked bleeding disorder caused by a deficiency of factor VIII (hemophilia A) or factor IX (hemophilia B). Coagulation factor replacement therapy of hemophilia may be complicated by the formation of inhibitory or neutralizing antibodies (inhibitors). This side-effect occurs in approximately 25% of the patients with severe hemophilia A, and in about 5% of the patients with mild hemophilia A. The current focus of our studies comprises the immune recognition and processing of factor VIII by antigen-presenting cells. We have shown that factor VIII is rapidly internalized by antigen-presenting cells. Competition experiments using a set of defined monoclonal antibodies revealed that the C1 domain of factor VIII directs its uptake by antigen-presenting cells (Figure 2). Infusion of a monoclonal antibody directed towards the C1 domain delayed the immune response to factor VIII in a murine model for hemophilia. Together, these observations emphasize the physiological importance of C1-domain mediated endocytosis of factor VIII by antigen-presenting cells.

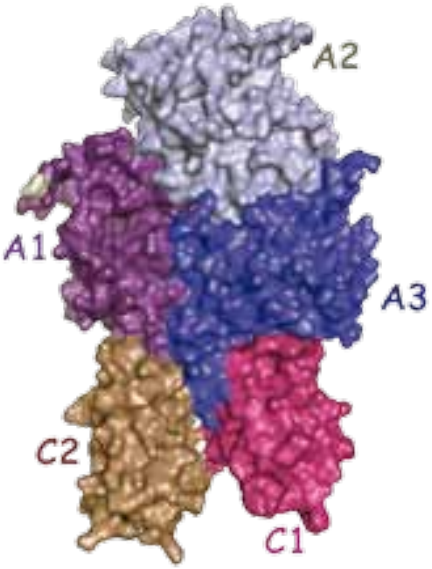

Figure 2: Domain structure of factor VIII. Our findings show that the C1 domain directs the uptake of factor VIII by antigen-presenting cells.

Thrombotic thrombocytopenic purpura

Thrombotic thrombocytopenic purpura (TTP) is a micro-angiopathy that is related to an acquired or congenital deficiency of the von Willebrand Factor (VWF) cleaving protease ADAMTS13. In the absence of ADAMTS13, ultra large VWF (UL-VWF) polymers, originating from endothelial cell specific organelles, designated Weibel-Palade bodies, accumulate in the circulation. These UL-VWF polymers mediate the formation of platelet-rich thrombi in the microcirculation that give rise to hemolytic anemia and thrombocytopenia. In plasma of the majority of patients with acquired TTP, antibodies directed towards ADAMTS13 are present. The majority of patients develop antibodies directed towards the spacer domain of ADAMTS13. In a recent study we have shown that the exposed surface in the spacer domain, comprising residues R568, F592, R660, Y661 and Y665, contributes to the binding of anti-ADAMTS13 antibodies (Figure 3). Our findings suggest that human antibodies directed towards this exposed surface in the spacer domain interfere with the productive assembly of the VWF-ADAMTS13 complex, thereby interfering with the cleavage of ultra-large VWF multimers on the surface of endothelial cells.

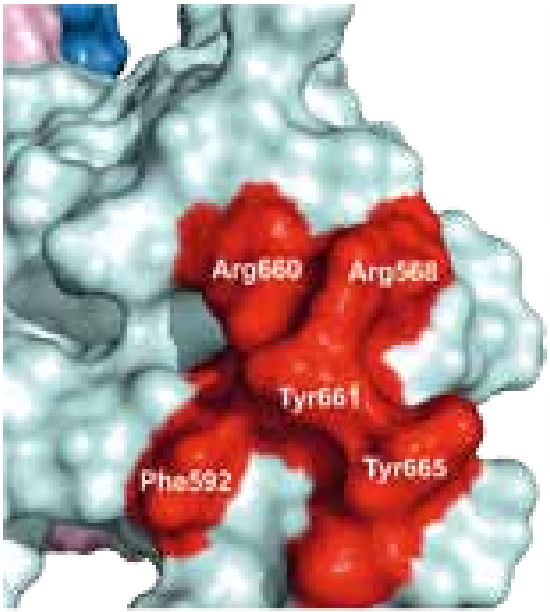

Figure 3: Exposed surface containing residues R568, F592, R660, Y661 and Y665 (indicated in red) in the spacer domain of ADAMTS13 that provides a binding site for pathogenic anti-ADAMTS13 antibodies that develop in patients with TTP.

Key publications

Pos W, Sorvillo N, Fijnheer R, Feys HB, Kaijen PH, Vidarsson G, Voorberg J. Residues Arg568 and Phe592 contribute to an antigenic surface for anti-ADAMTS13 antibodies in the spacer domain. Haematologica 2011; 96:1670-7.

Pos W, Crawley JT, Fijnheer R, Voorberg J, Lane DA, Luken BM. An autoantibody epitope comprising residues R660, Y661 and Y665 in the ADAMTS13 spacer domain identifies a binding site for the A2 domain of VWF. Blood 2010; 115:1640-9.

Herczenik E, van Haren SD, Wroblewska A, Kaijen PH, van den Biggelaar M, Meijer AB, Martinez-Pomares L, ten Brinke A, Voorberg J. Uptake of blood coagulation factor VIII is mediated via its C1 domain. J Allergy Clinical Immunol 2012; 129:501-9.

Van Haren SD, Wroblewska A, Fischer K, Voorberg J, Herczenik E. Requirements for immune recognition and processing of factor VIII by antigen-presenting cells. Blood Rev 2012; 26:43-9.

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Hematopoiesis

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Hematopoiesis is the process by which mature peripheral blood cells are formed from the hematopoietic stem cell. This process involves maintenance of the stem cell compartment, commitment of multipotent progenitors into the various lineages of the blood cell system, transient amplification of progenitor pools, and maturation to functional cells that are released into the circulation.

The Department of Hematopoiesis aims to be an expertise center for basic research on the survival, maintenance and lineage commitment of hematopoietic stem and progenitor cells, and on the maturation of progenitors into functional peripheral blood cells. Whenever possible, we will use this knowledge for the development of novel cellular products that could be produced by the stem cell laboratory.

Research lines:

- Bone marrow microenvironment
- The role of Slit-Robo signaling in hematopoiesis
- Megakaryopoiesis and Erythropoiesis
- Molecular analysis of T cell differentiation and memory formation.

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Bone marrow microenvironment

Matrix protein βig-h3 function in hematopoietic and stromal cells
We investigated the role of βig-h3 in human hematopoiesis postulating that βig-h3 may control homeostasis and the regenerative capacity of HSC self-renewal and differentiation.
We observed high expression of βig-h3 in bone marrow stromal cells, whereas expression in hematopoietic progenitors was low and increased as cells differentiate to monocytes. Most βig-h3 was excreted, but we also detected a distinct intracellular staining.
Overexpression of βig-h3 in hematopoietic stem- and progenitor cells (HSPC) accelerated megakaryopoiesis and increased the percentage of mature megakaryocytic cells. In contrast, granulocytic proliferation and the number of early colony-forming-unit-granulocyte-monocyte (CFU-GM) progenitors were reduced, while the erythrocytic differentiation was not affected. Together these data indicate that βig-h3 functions differentially in distinct hematopoietic lineages.
Knock-down of βig-h3 in HSPC resulted in a significant drop of CFU-GM formation and a small, but significant, decrease in the number of colony-forming-unit-erythrocyte, which is explained by reduced proliferation of βig-h3 knock-down cells. Accordingly, knock-down of βig-h3 resulted in reduced cell numbers in HSPC and stromal cell cultures associated with a reduction in the percentage of cycling cells and reduced levels of cell cycle genes. Together these data indicate that βig-h3 plays a role in cell proliferation.
Interestingly, knock down of βig-h3 in HSPC increased the potential of HSPC to form cobblestone-areas under a layer of stromal feeder cells. The increased number of cobblestone-area-forming-cells detected at week 4 and 6 in this assay indicated that reduction of βig-h3 levels maintains the renewal capacity of HSPC.
This PhD project also involves studying the role of βig-h3 in adhesive interactions and migration of HSPC and its role in hematopoietic malignancies.

Role of mesenchymal stromal cells (MSC)

MSC are a potential cell source for cellular therapies, for which recruitment and migration of MSC towards injured tissue is crucial. However, culture-expanded MSC contain only a small percentage of migrating cells *in vitro* (Maijenburg et al., 2009). To identify genes involved in the process of MSC migration, we generated gene expression profiles of migrating and non-migrating fetal bone marrow MSC (FBMSC). The nuclear receptors Nur77 and Nurr1 showed the highest expression in migratory MSC. Expression of Nur77 and Nurr1 was rapidly increased upon exposure of FBMSC to the migratory stimuli stromal-derived factor-1α (SDF-1α) and platelet-derived growth factor-BB. Lentiviral expression of Nur77 or Nurr1 enhanced migration of FBMSC toward SDF-1α compared with mock-transduced FBMSC and decreased the proportion of cells in S-phase compared with control cells. Further, gain-of-function experiments showed increased hepatocyte growth factor expression and interleukin (IL)-6 and IL-8 production in MSC. Despite the altered cytokine profile, FBMSC expressing Nur77 or Nurr1 maintained the capacity to inhibit T cell proliferation in a mixed lymphocyte reaction. Our results demonstrate that Nur77 and Nurr1 promote FBMSC migration. Modulation of Nur77 and Nurr1 activity may therefore offer perspectives to enhance the migratory potential of FBMSC which may specifically regulate the local immune response.
In addition, we studied the isolation and use of primary MSC (without *ex vivo* expansion) and demonstrated that CD271 and CD146 define distinct colony-forming-unit-fibroblasts containing mesenchymal stromal cell subpopulations. Analysis of 86 bone marrow samples revealed that the distribution of $CD271^{bright}CD146^{-}$ and $CD271^{bright}CD146^{+}$ subsets correlates with donor age. The main subset in adults was $CD271^{bright}CD146^{-}$, whereas the $CD271^{bright}CD146^{+}$ population was dominant in pediatric and fetal bone marrow. A third subpopulation of $CD271^{-}CD146^{+}$ cells contained colony-forming-unit-fibroblasts in fetal samples only. These changes in composition of the mesenchymal stromal cell compartment during development and aging suggest a dynamic system, in which these subpopulations may have different functions.
Interestingly, we observed clear differences in Wnt-(target) gene expression between the primary and cultured adult MSC subsets and between Adult BMSC and Fetal BMSC, which correlated with differences in hematopoietic support. ABMSC and FBMSC differed in Wnt5a expression, but also in the response to exogenous Wnt3a. The distinct response to inhibition of endogenous Wnt-production may be explained by variation in expression of Wnt-inhibitors and Frizzled receptors on the two MSC sources. This seems to lead to a different net balance in autocrine Wnt-signaling between these cells, established through distinct intracellular mechanisms. These last two topics are now studied in the current PhD project.

Key Publications:
Maijenburg MW, Gilissen C, Melief SM, Kleijer M, Weijer K, ten Brinke A, Roelofs H, van Tiel CM, Veltman JA, de Vries CJ, van der Schoot CE, Voermans C. Nuclear receptors nur77 and nurr1 modulate mesenchymal stromal cell migration. Stem Cells Dev 2012; 21(2):228-38.

Maijenburg MW, van der Schoot CE, Voermans C. Mesenchymal stromal cell migration: possibilities to improve cellular therapy. Stem Cells Dev 2012; 21(1):19-29.

Maijenburg MW, Kleijer M, Vermeul K, Mul EP, van Alphen FP, van der Schoot CE, Voermans C. The composition of the mesenchymal stromal cell compartment in human bone marrow changes during development and aging. Haematologica 2012; 97(2):179-83.

Maijenburg MW. Characterization of human mesenchymal stromal cell hetereogeneity. Thesis October 12, 2011. University of Amsterdam.

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The role of Slit-Robo signaling in hematopoiesis

Slit stimulates erythropoiesis

Slit extracellular matrix proteins are expressed by bone marrow (BM) stromal cells, whereas their receptors, the Roundabout (Robo) proteins, are expressed by hematopoietic stem and progenitor cells (HSPC). We previously showed that homing of HSPCs to the BM was enhanced when the HSPCs were pre-treated with Slit3. Currently, we investigate whether and by what molecular mechanism Slit3 affects the proliferation and differentiation of HSPCs. Slit3 did not change the number of colony-forming units in the granulocyte-monocyte lineage or the megakaryocytic lineage, but increased the number of the burst-forming unit erythroid (BFU-E) progenitors. BFU-E colony formation from sorted hematopoietic stem cells, common myeloid progenitors and megakaryocyte-erythrocyte progenitors increased in the presence of Slit3, but BFU-E colonies did not grow from the granulocyte-monocyte progenitors fraction.
When HSPCs were cultured in the presence or absence of Slit3 in serum-free liquid medium containing TPO, SCF and Flt3, we observed that Slit3 increased the total number of nucleated cells, and the number of cells with an immature erythroblast phenotype ($CD34^{-}CD45^{-}CD36^{+}CD71^{+}$), while also the frequency of the cells able to form BFU-E was increased.
Concerning the molecular mechanism, we found that Robo1 interacts with the adaptor proteins Nck and p130Cas. Slit3 decreased the tyrosine phosphorylation of Robo1 and increased the tyrosine phosphorylation of p130Cas, resulting in recruitment of the tyrosine kinase Lyn, member of the Src family of tyrosine kinases and implicated in the regulation of erythropoiesis, to the Robo complex.
Together, our data indicate that Slit3 promotes expansion of the erythroid progenitor compartment which results in an increased output of erythroid cells. Downstream of Slit3 this may involve activation of the Lyn kinase.

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Megakaryopoiesis and Erythropoiesis

The role of transcription factor MEIS1

This research line focuses on the role of the homeobox transcription factor MEIS1 in human hematopoiesis. We previously developed a lentiviral system that allows us to over-express and knock-down MEIS1 in human hematopoietic stem and progenitor cells. Using this method, we discovered that expression of MEIS1 is indispensable for the megakaryocyte/erytroid lineage decision and we found evidence that the effects of MEIS1 are mediated by GATA-1. We also showed that MEIS1 is a positive regulator of proliferation in megakaryocytes and erythrocytes. Since MEIS1 is specifically upregulated during megakaryopoiesis, we aim to study which genes are regulated by MEIS1 and how this might affect platelet production and functionality.

Key publications:

O'Conner MN, Thijssen-Timmer DC, Broos K, Deckmyn H. Platelet Proteomics, Chapter 11: Platelet Functional Genomics, Salles II, 12 July 2011, DOI: 10.1002/9780470940297.Ch11.

Differentiation of erythroblasts to erythrocytes

Differentiation of erythroblasts to erythrocytes involves the assembly of the plasma-membrane band 3 macro-complex. This complex consists of over 20 proteins some of which are important blood group antigens, e.g. Rh, Aquaporin, band 3, GPA, LW, and Kell. Key functions of this protein complex include the regulation of deformability through protein 4.2 and ankyrin-dependent association to the underlying spectrin cytoskeleton, bicarbonate/chloride exchange as a function of erythrocyte CO_2 transport and pH regulation, and RhAG-mediated ammonia transport. Mutations in proteins of the band 3 macro-complex can lead to specific hemolytic anemias of variable severity. Using a human erythroblast culture, we found that the effects of these mutated proteins are already apparent during early erythropoiesis and have repercussions on macro-complex assembly, possibly affecting functionality. Hence it became important to research the assembly of these erythrocyte membrane protein complexes during normal erythroblast differentiation. In 2011 we published the spatio-temporal assembly of the band 3 macro-complex by following the interaction, synthesis and intracellular routing of several proteins: 4.2/band 3 and RhAG/Rh. Good knowledge about the assembly of the band 3 complex and its association with the underlying cytoskeleton is crucial for our understanding of hemolytic disease, blood group presentation and general erythrocyte functionality. We are currently investigating the signals necessary to optimize reticulocyte maturation to erythrocytes and how post-translation modifications like phosphorylation can influence this process.

Key publication

Satchwell TJ, Bell AJ, Pellegrin S, Kupzig S, Ridgwell K, Daniels G, Anstee DJ, van den Akker E*, Toye AM*. Critical band 3 multiprotein complex interactions establish early during human erythropoiesis. Blood 2011; 118(1):182-91. *contributed equally to this work.

Role of mRNA translation in erythropoiesis

Whereas transcription factors lay out the basic gene expression program, a cell's proteome is determined by many post-transcriptional processes among which is mRNA translation. Marieke von Lindern previously showed how growth factor signaling controls the translation of transcripts with long 5'-untranslated regions and a complex secondary structure. We thought that these transcripts were also affected in Diamond Blackfan Anemia (DBA), a disease with congenital mutations in ribosomal proteins. This was not the case. Instead we found impaired translation of transcripts translated from an internal ribosomal entry site (IRES). Among them are Bag1 (Bcl2 athanogene 1) and Csde1 (cold shock domain protein e1) that are 20 and 200-fold upregulated, respectively, when hematopoietic progenitors differentiate to erythroblasts. Translation of Bag1 and Csde1 is also impaired in erythroblasts cultured from blood of DBA patients. Mice lacking Bag1 die before birth due to a lack of mature erythrocytes. Lack of Bag1 increases the phosphorylation of translation initiation factor 2, which may particularly affect the translation of transcripts with upstream open reading frames. Knockdown of Csde1 severely impairs erythroid proliferation and differentiation. Csde1 is an IRES transactivating factor. We are currently identifying the transcripts that depend on Bag1 or Csde1 for translation to identify the mechanisms that are affected.

Key Publication

Horos R, Ljspeert H, Pospisilova D, Sendtner R, Andrieu-Soler C, Taskesen E, Nieradka A, Cmejla R, Sendtner M, Touw IP, von Lindern M. Ribosomal deficiencies in Diamond-Blackfan anemia impair translation of transcripts essential for differentiation of murine and human erythroblasts. Blood 2012; 119(1):262-72.

Monika Wolkers PhD, m.wolkers@sanquin.nl

Molecular analysis of T cell differentiation and memory formation

The molecular regulation of T cell memory

CD8 memory T cells are crucial to protect us from recurring infections. When they re-encounter the pathogen, T cells get swiftly reactivated which allows for rapid generation of effector molecules and clearance of infected cells. For the generation of proficient CD8 T cell memory, survival signals from CD4 T helper cells are required. Lack of CD4 T cell help results in tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated cell death of CD8 T cells upon reactivation. In our recent studies, we have dissected the molecular mechanisms on how Interleukin-2 rescues 'helpless' CD8 T cells (Wolkers et al., Immunol Lett 2011). Importantly, we found that 'help' is imprinted in CD8 T cells through the transcriptional regulator Nab2, which suppresses TRAIL expression in helped CD8 T cells, thereby allowing for the development of proficient secondary CD8 T cell responses (Wolkers et al., Blood 2011).

Regulation of TRAIL expression in plasmacytoid DCs and NK cells

Given that TRAIL is also employed by immune cells to specifically kill virally infected and cancer cells, we addressed whether Nab2 also affects TRAIL expression in other cell types. Our preliminary data showed that TRAIL expression in natural killer (NK) cells is also regulated by Nab2 (Balzarolo et al. manuscript in preparation). Conversely, we found that Nab2 is required for TRAIL expression in plasmacytoid dendritic cells (pDCs). We further unraveled the Nab2-mediated TRAIL induction and established that activated pDCs require the engagement of two signaling pathways for optimal TRAIL expression, i.e. for optimal target cell killing: PI3K-Nab2 signaling and type I IFN-R engagement (Balzarolo et al., manuscript under review).

The molecular regulation of T cell effector functions

One hallmark of CD8 memory T cells is that massive production of effector molecules is ensured within a few hours upon reactivation. This high responsiveness of memory T cells correlates with elevated levels of cytokine transcripts that allow for rapid generation of the effector molecules. Strikingly, regardless of these high mRNA levels, cytokine production is blocked unless the cognate antigen is recognized, thereby preventing uncontrolled protein production of otherwise toxic molecules. We have recently developed a model system to study how this translational block is mediated, and found that both *in vitro* and *in vivo*, the effector molecule interferon (IFN) γ is regulated through translational regulation. We have identified the sequences required for this translational block (Salerno et al.; manuscript in preparation). We are currently investigating which proteins are involved in the translational regulation and aim to understand the functional consequence for this translational regulation.

Key publications

Hennies CM, Reboulet RA, Garcia Z, Nierkens S, Wolkers MC, Janssen EM. Selective expansion of merocytic dendritic cells and CD8DCs confers anti-tumor effect of Fms-like tyrosine kinase 3-ligand treatment in vivo. Clin Exp Immunol 2011; 163:381-91.

Wolkers MC, Bensinger SJ, Green DR, Schoenberger SP, Janssen EM. Interleukin-2 rescues helpless effector CD8+ T cells by diminishing the susceptibility to TRAIL- mediated death. Immunol Lett 2011; 139(1-2):25-32.

Wolkers MC, Gerlach C, Arens R, Janssen EM, Fitzgerald P, Schumacher TN, Medema JP, Green DR, Schoenberger SP. Nab2 regulates secondary CD8+ T-cell responses through control of TRAIL expression. Blood 2012; 119(3):798-804.

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Laboratory of Adaptive Immunity

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In the beginning of 2011, Martijn Nolte moved with his research group from the Department of Experimental Immunology at the AMC to the Department of Hematopoiesis at Sanquin, where he founded the Laboratory for Adaptive Immunity. On one hand this lab has a research interest in the impact of immune activation on hematopoiesis, and on the other, in close collaboration with Prof van Lier, in the molecular mechanisms that underlie the formation of effector and memory T cells. As such, this lab operates at the crossroads of hematology and immunology and is now fully integrated in the Department of Hematopoiesis.

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The impact of immune activation on hematopoiesis

With the aim of investigating the impact of immune activation on hematopoiesis we have found that activated T cells in the bone marrow (BM) are able to provide feedback signals during the course of a viral infection. We found that activated T cells in the BM can inhibit the production of both eosinophilic (De Bruin et al., 2010) and neutrophilic granulocytes (De Bruin et al., 2012) through the production of the pro-inflammatory cytokine interferon-gamma (IFNγ). Molecular analysis revealed that IFNγ-treatment of myeloid precursors negatively affects the signaling pathways downstream of the IL-5R and the G-CSFR, which are essential for the formation of eosinophilic and neutrophilic granulocytes, respectively. Conversely, IFNγ rather enhanced the production of monocytes by upregulating the monocyte-inducing transcription factors IRF-8 and PU.1 (De Bruin et al., 2012). These findings illustrate that IFNγ-producing T cells are important mediators in shaping the hematopoietic response during inflammation, as they promote the production of the appropriate myeloid cell type upon viral infection and simultaneously suppress formation of cells that are less important for anti-viral defense.

Apart from the impact of IFNγ on hematopoiesis in the short term, we found that sustained production of IFNγ induces a progressive form of anemia, which could be attributed to a reduction in the lifespan as well as the formation of red blood cells. The decrease in erythrocyte half-life could be explained by an IFNγ-induced activation of macrophages in the splenic red pulp (Libregts et al., 2011). We have demonstrated that IFNγ induces expression of the transcription factor IRF-1, which subsequently binds to the promoter of PU.1 and induces PU.1 expression, leading to inhibition of erythropoiesis. Notably, down regulation of either IRF-1 or PU.1 expression was sufficient to overcome IFNγ-induced inhibition of erythropoiesis (Libregts et al., 2011).
The findings described above illustrate how T cells in the BM can have a strong impact on the formation of new blood cells in the BM, through the production of IFNγ (Figure 1). Although a temporary shift in hematopoiesis might be beneficial during acute viral infections, the prolongation of such a shift can lead to the development of anemia, which is frequently observed in patients suffering from chronic inflammatory diseases, such as HIV-infection and rheumatoid arthritis. Moreover, a prolonged blockade in the formation of eosinophilic and neutrophilic granulocytes will also impair anti-bacterial responses, which could explain the increased vulnerability to bacterial infections that occurs after a viral infection.

Key Publications:

De Bruin AM, Libregts SF, Valkhof M, Boon L, Touw IP, Nolte MA. Interferon-gamma induces monopoiesis and inhibits neutrophil development during inflammation. Blood 2012; 119(6):1543-54.

Libregts SF, Gutiérrez L, de Bruin AM, Wensveen FM, Papadopoulos P, van IJcken W, Özgür Z, Philipsen S and Nolte MA. Chronic IFNγ production in mice induces anemia by reducing erythrocyte lifespan and inhibiting erythropoiesis through an IRF-1/PU.1-axis. Blood 2011; 118(9):2578-88.

De Bruin AM, Buitenhuis M, van der Sluijs KF, van Gisbergen KP, Boon L, Nolte MA. Eosinophil differentiation in the bone marrow is inhibited by T cell-derived IFN-gamma. Blood 2010; 116(14):2559-69.

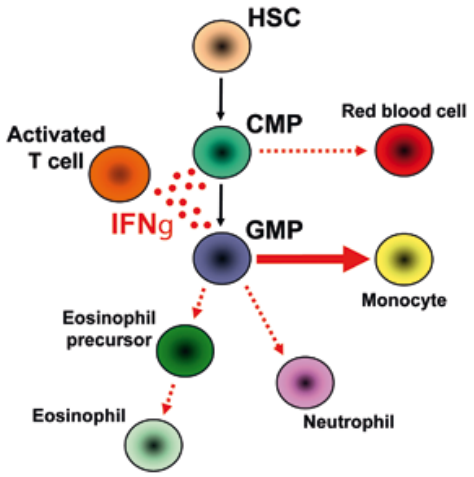

Figure 1:
Our data demonstrate that IFNγ produced by activated T cells can dramatically alter the hematopoietic differentiation in the bone marrow. IFNγ was shown to negatively affect differentiation towards both eosinophilic and neutrophilic granulocytes, as well as red blood cells (red dotted arrows), whereas it strongly enhances the formation of monocytes (large red arrow).

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The molecular mechanism of effector T cell formation

The unifying theme of this research line is the regulation of effector/memory T cell formation in humans and mice (Figure 2). The goals are to determine the contribution of costimulatory molecules and transcription factors in the differentiation towards effector T cells and to identify functionally distinct T cell subsets and their role in normal and pathophysiological immune reactions. Regarding the latter, we have found that the human lung harbors a resident subset of $CD8^+$ T cells expressing CD103 (aE integrin) and that this subset is highly enriched for $CD8^+$ T cells specific to respiratory viruses, such as influenza, but not to systemic viruses, such as the Epstein Barr virus and the cytomegalovirus. $CD103^+CD8^+$ T cells produced large amounts of IFNγ, but did not contain perforin nor granzyme B, which indicates that this pool of resident $CD8^+$ T cells can provide a rapid response to viral infection without inducing cytotoxic damage to the delicate epithelial barrier (Piet et al., 2011). Following a long-standing interest in the consequences of T cell costimulation through CD27 and its ligand CD70, we have recently found that CD27 triggering on $CD4^+$ T cells does not provide instructive signals for a specific $CD4^+$ T cell subset, but, depending on the cytokine milieu and genetic background, supports Th1 cell formation, while it inhibits the formation of Th17, but not Th2 cells (Libregts et al., Imm Letters 2011). Moreover, we established that CD27-driven costimulation lowers the threshold of T cell receptor activation for $CD8^+$ T cells and enables responses against low-affinity antigens. We therefore propose that CD27-driven costimulation is a strategy to generate memory clones that have potential reactivity to a wide array of mutable pathogens (Van Gisbergen et al., 2011).
Finally, based on transcriptome analysis of primary effector $CD8^+$ T cells in humans, we have identified a novel molecule, ZNF683, that is strongly upregulated in effector CD8 T cells (Hertoghs et al., J Clin Invest 2010). The expression profile of ZNF683 suggests that this factor is involved in modulating CD8 T cell differentiation. Based on its high homology to the transcription factor Blimp-1, we have renamed ZNF683 as Hobit, for Homologue of Blimp-1 in T cells. Expression analysis in humans revealed that Hobit is mainly expressed in effector CD8 T cells, NK cells and cytotoxic CD4 T cells. Interestingly, this pattern was markedly different in mice, where we found Hobit to be expressed predominantly in NKT cells. Detailed analysis of Hobit-deficient mice indicated that Hobit is required for terminal differentiation of NKT cells and for the stimulus-induced cytolytic effector function. Based on our findings we postulate that Hobit is a novel master regulator of cytotoxicity in lymphocytes.

Publications:

Wensveen FM, Derks IA, van Gisbergen KP, de Bruin AM, Meijers JC, Yigittop H, Nolte MA, Eldering E, and van Lier RA. BH3-only protein Noxa regulates apoptosis in activated B cells and controls high-affinity antibody formation. Blood 2012; 119(6):1440-9.

Van Gisbergen KP, Klarenbeek PL, Kragten NA, Unger PP, Nieuwenhuis MB, Wensveen FM, ten Brinke A, Tak PP, Eldering E, Nolte MA, van Lier RA. The costimulatory molecule CD27 maintains clonally diverse CD8 (+) T cell responses of low antigen affinity to protect against viral variants. Immunity 2011; 35(1):97-108.

Piet B, de Bree GJ, Smids-Dierdorp BS, van der Loos CM, Remmerswaal EB, von der Thüsen JH, van Haarst JM, Eerenberg JP, ten Brinke A, van der Bij W, Timens W, van Lier RA, Jonkers RE. CD8+ T cells with an intraepithelial phenotype upregulate cytotoxic function upon influenza infection in human lung. J Clin Invest 2011; 121(6):2254-63.

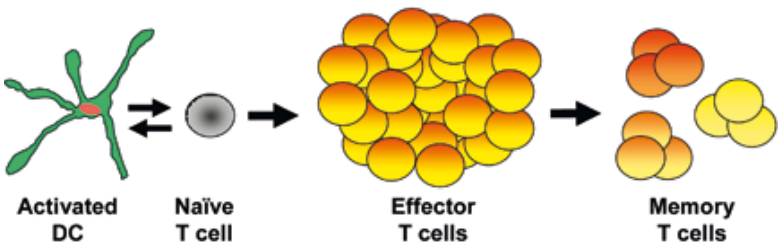

Figure 2:
Differentiation of naive T cells towards effector and memory T cells is strongly dependent on the interactions of the naive T cell with the activated dendritic cell that presents the cognate antigen. Our lab investigates the underlying molecular mechanism that regulates the quantity and quality of the ensuing T cell response.

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Experimental Immunohematology

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Research of the Department of Experimental Immunohematology is focused on the immunohematological aspects of cellular therapy. We are studying the immune responses to classical blood products such as red cells and platelets, and perform research on the development of new cellular products.
The department also accommodates the Immunocytology diagnostic sub-laboratory and the certified Laboratory for Stem Cell Transplantation

The research is embedded in the following research lines:

- Immune response to blood group antigens:
  – new methods to type blood group antigens by genetic and proteomic approaches
  – immune responses against Blood Cells
- Detecting minimal residual disease in childhood cancers
- New cellular therapies

These research lines all have various subprojects that share some fundamental aspects – both within the group and with various other groups within Sanquin, the Academic Medical Center in Amsterdam and other research institutes and biotech companies, as outlined in the corresponding paragraphs.

Immune response to blood group antigens

The aim of this research line is to develop new diagnostic and preferentially also therapeutic options to further prevent and/or treat allo- or autoimmunization against blood cells. We are studying both the antigens, which are the targets of the immune response, and the humoral immune response that leads to cell destruction.

Blood group antigens

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In the past this research line focused on the biochemical and molecular characterization of platelet antigens. In later years the focus moved to the molecular characterization of red blood cell antigens, especially Rhesus (Rh). We now focus on new techniques for mass-scale red cell genotyping, and phenotyping, and aim to unravel the molecular background of high-frequency red cell antigen systems. The ultimate goal of our research is to change transfusion policy. At present, blood transfusion is only matched for ABO and RhD, and the donor is screened for the presence of red cell antibodies. By making available: 1) cost effective genotyping of both donors and recipients and 2) insight into risk factors for alloimmunization (genetic factors as well as disease-related), new algorithms for transfusion can be developed. Selected patient groups can be transfused with matched blood cell products. This will cause shifting from lab-based selection of blood products to electronic matching.

Ad 1) Using a Multiplex Ligase probe Amplification (MLPA) genotyping assay that we developed in collaboration with MRC-Holland, we are currently able to type 52 blood group antigens of 18 blood group systems and two platelet systems (HPA1 and HPA2). Since genotyping assays are hampered by relatively high costs and the presence of rare or even unknown null-alleles, we are currently also making an effort to set up unique proteomic-based and label-free assays for typing red blood cell antigens directly.
In 2011 non-invasive fetal RhD typing was introduced in the Netherlands to guide both antenatal and postnatal anti-D prophylaxis in D-negative pregnant women. In the first year all cord blood samples are sent to Sanquin, and the program will be evaluated.

Ad 2) To identify genetic risk factors for alloimmunization, we are continuously banking DNA samples of red cell alloimmunized pregnant women for a genome-wide association study (GWAS). The implementation of samples is nearly finished and in collaboration with Prof WH Ouwehand, Cambridge, over 2000 samples will be studied. The DNA bank of healthy Dutch donors (the Sanquin Control Cohort), which will serve as the control in our study, has already been used by several other GWAS projects and is available for research groups upon request.

Key publications:

Scheffer PG, de Haas M, van der Schoot CE. The controversy about controls for fetal blood group genotyping by cell-free fetal DNA in maternal plasma. Curr Opin Hematol 2011; 18(6):467-73. Review.

Vergeer M, Boekholdt SM, Sandhu MS, Ricketts SL, Wareham NJ, Brown MJ, de Faire U, Leander K, Gigante B, Kavousi M, Hofman A, Uitterlinden AG, van Duijn CM, Witteman JC, Jukema JW, Schadt EE, van der Schoot E, Kastelein JJ, Khaw KT, Dullaart RP, van Tol A, Trip MD, Dallinga-Thie GM. Genetic variation at the phospholipid transfer protein locus affects its activity and high-density lipoprotein size and is a novel marker of cardiovascular disease susceptibility. Circulation 2010; 122(5):470-7

Humoral immune response

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In most immune-mediated blood cell diseases and in all fetal/neonatal alloimmune cytopenias the destruction of blood cells is mediated by antibodies. We are therefore investigating both 1) the B cells, 2) the characteristics of these antibodies and 3) the interaction of antibodies with the FcRn, the receptor responsible for placental transport.

B cells

In a previously developed culture method in which B cells are stimulated to Immunoglobulin (Ig) production at the single cell level, we found that in hyper immune anti-D donors the majority of antigen-specific memory cells resides in the IgM-positive B cells. Upon antigen challenge the number of IgG-positive cells increased, whereas the IgM positive cells remained stable. We have now shown that these IgM memory cells are mostly CD27-negative, but harbor both mutations in the BCL-6 gene and in the variable regions of the VH and VL genes, proving that these cells are true memory cells. In analogy with recent studies in mice, we postulate that the IgM memory cells will not class-switch in the presence of IgG in the serum, but can replenish the memory pool once the titer has dropped and in case of infectious agents the antigenic make-up of the pathogen might have changed.

Antibodies

By analyzing the Fc-glycosylation of the IgG1 alloantibodies formed during pregnancy against antigens of the fetus (Human Platelet Antigen-1 or RhD) using mass spectrometry, we found markedly decreased levels of core-fucosylation and increased levels of galactosylation and sialylation as compared to total serum IgG1 of the same patients. Because IgG1 Fc-core-fucosylation influences antibody-dependent cell-meditated cytotoxicity activity, this may have a profound effect on disease severity and prognosis. To correlate Fc-glycosylation with biological activity we developed an assay to determine the induction of Fc-γ-receptor mediated respiratory burst by anti-HPA opsonized platelets. Remarkably, this assay was found to be dependent on the presence of C-reactive protein (CRP). This serum protein was increased in cord blood samples of fetal neonatal alloimmune thrombocytopenia affected neonates. In a murine model of Idiopathic Thrombocytopenic Purpura it was demonstrated that CRP enhances the antibody mediated breakdown of platelets (Figure 1).

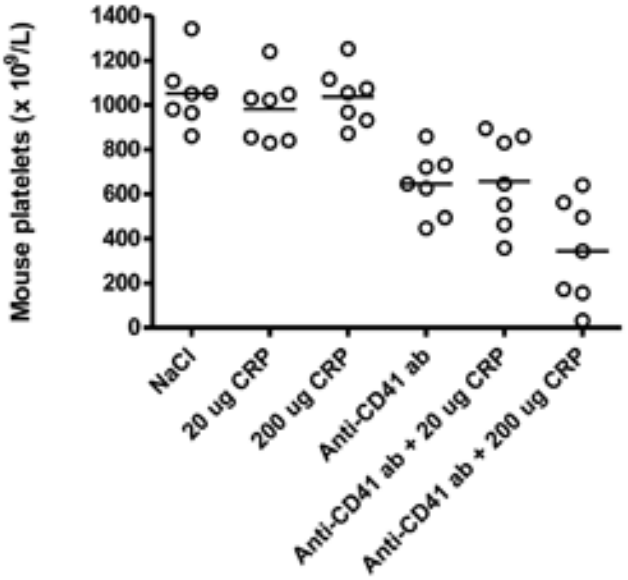

Figure 1: CRP contributes to platelet destruction induced by anti-platelet antibody in an *in vivo* mouse model of ITP. BALB/C mouse-platelet counts after injection of rat anti-mouse CD41 antibody are significantly decreased if 200 µg CRP is coinjected ($p<0.01$)

FcRn

Human IgG3 displays the strongest effector functions of all human IgG subclasses but has a short half-life, suggesting FcRn-mediated IgG salvage to be defective for IgG3.
We have previously observed that human IgG1 inhibited FcRn-mediated transport of IgG3 at the level of receptor binding. This inhibition was due to a single amino acid difference at position 435, where IgG3 has an arginine instead of the histidine. Importantly we showed that the half lives of natural H435-containing IgG3 allotypes in humans are comparable to IgG1. This H435-IgG3 also proved better suited for protection against pneumococcal challenge in mice, demonstrating that H435-IgG3 is a formidable candidate for monoclonal antibody therapies in patients.

Key publication:

Stapleton NM, Andersen JT, Stemerding AM, Bjarnarson SP, Verheul RC, Gerritsen J, Zhao Y, Kleijer M, Sandlie I, de Haas M, Jonsdottir I, van der Schoot CE, Vidarsson G. Competition for FcRn-mediated transport gives rise to short half-life of human IgG3 and offers therapeutic potential. Nature Commun 2011; 2:599.

Minimal residual disease detection in childhood cancers

The prognosis of cancers in childhood is often better than in adults, but yet many children do not survive. To recognize children who might benefit from other therapeutic strategies, in collaboration with GA Tytgat, HN Caron and R Versteeg (AMC/EKZ, Amsterdam), V de Haas (SKION), JJM van Dongen and VHJ van der Velden (Erasmus MC), we develop and evaluate assays for the detection of minimal residual disease in childhood cancers. We demonstrated that hyper-methylated RASSF1a can be used as a DNA marker for the detection of minimal residual disease for neuroblastoma.

Key publications:

Stutterheim J, Ichou FA, den Ouden E, Versteeg R, Caron HN, Tytgat GA, van der Schoot CE. Methylated RASSF1a is the first specific DNA marker for minimal residual disease testing in neuroblastoma. Clin Cancer Res 2012; 18(3): 808-14.

Waanders E*, van der Velden VH*, van der Schoot CE*, van Leeuwen FN, van Reijmersdal SV, de Haas V, Veerman AJ, van Kessel AG, Hoogerbrugge PM, Kuiper RP, van Dongen JJ. Integrated use of minimal residual disease classification and IKZF1 alteration status accurately predicts 79% of relapses in pediatric acute lymphoblastic leukemia. Leukemia 2011; 25(2):254-8. *These authors contributed equally to this work.

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Cellular therapies

In the past years several research projects within Sanquin have led to the development of new cellular products. Some of these products are now ready to be introduced into the clinic. Due to European legislation these cellular therapies are considered medicines which implies that they have to be manufactured under strict conditions. Therefore Sanquin has set up a facility (Sanquin Cellular Therapy Services) within the Laboratory for Stem Cell Transplantation to translate novel cellular products towards cellular therapies and to conduct clinical trials. Since we expect an increasing amount of work involving (new) cellular therapies, a new good manufacturing practice (GMP) facility has been built. This facility comprises four separate class B labs (one for ML-2 activities) and one class C lab.

In 2011 we have been working on three projects. The first project concerns dendritic cell (DC) immunotherapy for esophageal adenocarcinoma, for which we developed a clinical grade protocol in a closed culture system. Monocyte-derived DC from patients suffering from esophageal cancer are matured with a patented GMP maturation cocktail (consisting of MPLA/IFNγ) and loaded with tumor-derived mRNA. Patients will receive at least three DC vaccines via intradermal injection. This phase 1 clinical trial will be performed in collaboration with the Department of Gastroenterology and Hepatology of the Amsterdam Medical Center.

In the second project, Sanquin offers its services for two clinical trials initiated at the AvL/NKI by Prof T Schumacher and Prof J Haanen. In the first trial, melanoma patients are treated with adoptive therapy using tumor-specific T cells generated by the transfer of T cell receptor (TCR) gene. T cells isolated from patients are transduced with a retroviral vector encoding a Mart-1 specific TCR and cells will be re-infused upon short-term *ex vivo* culture. The second trial on adoptive therapy consists of culturing melanoma-reactive T cells from resected metastases *ex vivo* and administration of them to patients with high dose chemotherapy and interleukine-2 bolus.

In the third project, mesenchymal stromal cells (MSCs) derived from the bone marrow will be expanded to treat acute graft-versus-host disease (GvHD). The Laboratory for Stem Cell Transplantation will participate in a phase III clinical trial conducted by the Department of Immunohematology and Blood Transfusion of the LUMC in collaboration with the European Group of Blood and Bone Marrow Transplantation. The trial design involves allogeneic stem cell transplantation in patients with acute GvHD grade 2-4, who are refractory on steroid treatment. The patients will be randomized to receive MSCs or only standard immunosuppressive therapy.

Key publications:

Van der Laan AM, Hirsch A, Robbers LF, Nijveldt R, Lommerse I, Delewi R, van der Vleuten PA, Biemond BJ, Zwaginga JJ, van der Giessen WJ, Zijlstra F, van Rossum AC, Voermans C, van der Schoot CE, Piek JJ. A proinflammatory monocyte response is associated with myocardial injury and impaired functional outcome in patients with ST-segment elevation myocardial infarction: monocytes and myocardial infarction. Am Heart J 2012; 163(1):57-65.e2.

Hommes DW, Duijvestein M, Zelinkova Z, Stokkers PC, Ley MH, Stoker J, Voermans C, van Oers MH, Kersten MJ. Long-term follow-up of autologous hematopoietic stem cell transplantation for severe refractory Crohn's disease. J Crohns Colitis 2011; 5(6):543-9.

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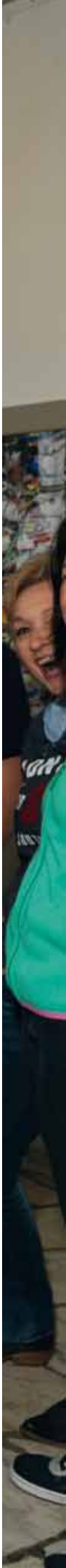

Immunopathology

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The Department of Immunopathology investigates the regulation of inflammation and tolerance against non-infectious antigens, with a specific focus on humoral immune responses. The research lines can be divided into five main themes. These lines are presented in the PI-pages of Lucien Aarden and Marieke van Ham, but are strongly interconnected.

- **The B cell research group** (Prof Marieke van Ham PhD) focuses on the role of antigen presentation by B cells in the regulation of the $CD4^+$ T helper cell response and the humoral immune response. Antigen-specific B cells that phagocytose particulate antigen and their interaction with specific $CD4^+$ T cells are investigated, as B cells play a central role in $CD4^+$ T cell reactivation and may direct T cell help towards antibody production.
- **The dendritic cell (DC) research group** (Anja ten Brinke PhD, Prof Marieke van Ham PhD) studies the regulation of DC effector functions during immune activation and immunological tolerance with the aim of developing clinically-approved monocyte-derived DC products. Next to classical DC maturation pathways, modulation of DC function through complement, Fc activation and immune complexes are investigated.
- **The immunoglobulin research group** (Theo Rispens PhD, Gertjan Wolbink MD PhD, Prof Lucien Aarden PhD) aims to understand the structure-function relationships of different human immunoglobulins. Research focuses specifically on the IgG4 B cell response, immune modulation by IVIg (intravenous immunoglobulins) and mechanisms and adverse effects of antibody formation to therapeutic antibodies.

Related research lines presented by Prof Lucien Aarden are:

- Complement research
- Inflammation

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Regulation of acquired immunity by antigen-specific B cells

Our research focuses on the role of antigen-specific B cells in B-T cell interactions and humoral immunity. We previously demonstrated that antigen-specific B cells phagocytose particles and bacteria. We now investigate how this leads to B cell differentiation, class switching and antibody formation. We make use of infection and cancer models and use tetanus toxoid and the therapeutic antibody Adalimumab to investigate antibody response against non-infectious antigens upon frequent antigen re-encounter (hyper-immunization or repeated treatment).

Antigen-specific B-T cell interactions and antibody production

Phagocytosis of *Salmonella typhimurium* by B cells leads to efficient CD4⁺ T helper (Th) cell activation, which in turn enhances *Salmonella*-specific antibody formation. B cells regulate CD4⁺ T cell polarization. During antigen recall, Th17 cells are activated. The induction of Th17 from naïve CD4⁺ T cells in humans is still unclear. We demonstrated that, compared to classical CD28 costimulation, alternative costimulation via CD5 is superior for human Th17-priming (Figure 1). We showed that this depends on elevation of pSTAT3 and IL-23 receptor expression. Whereas in mice Th17 cells coproduce IL-21, in humans other Th subsets produce IL-21. As IL-21 is a key factor for B cell help, we are currently studying if and how B cells induce IL-21 Th polarization.

B-T cell interactions and antibody production upon hyper-immunization

This year we focused on the formation of anti-tetanus toxoid (TT) antibodies in voluntary donors that are frequently boosted to obtain sufficient antibody titers. We established a well-defined cohort of donors with a confirmed history of frequent re-immunizations and documented specific antibody titers over 5-10 years, to investigate hyper-immunization effects on long-term humoral immunity. We performed frequent antibody titer measurements within one year of follow-up to also investigate effects on short-term antibody responses. *In vitro* B-T cell co-cultures indicate that frequent TT boosting induces CD4⁺ T cell subsets that are specialized in supporting antibody formation.

Antigen presentation in leukemia.

We are studying the regulation of MHC class I and II-Ag presentation in leukemia to determine whether tumor cells target these pathways to escape from immune recognition. In collaboration with Dr Arjan van de Loosdrecht (Department of Hematology, VUmc), we demonstrated last year that leukemic blasts use the MHC I chaperone TAP for peptide loading of MHC class II. This year we identified another form of cross-over of MHC class I and II regulation in acute myeloid leukemia. CLIP, an essential chaperone intermediate of MHC II presentation, also binds to MHC class I molecules and may lead to aberrant MHC I antigen presentation.

Key publications:

Paul P, van den Hoorn T, Jongsma M, Bakker M, Hengeveld R, Cresswell P, Egan D, van Ham M, ten Brinke A, Ovaa H, Beijersbergen R, Kuijl C, Neefjes J. Genome-wide siRNA screen identifies novel pathways controlling MHC class II antigen presentation. Cell 2011; 145:268-83.

De Wit J, Souwer Y, van Beelen AJ, de Groot R, Muller FJ, Klaasse Bos H, Jorritsma T, Kapsenberg ML, de Jong EC, van Ham SM. CD5 costimulates for stable human Th17 development by promoting IL-23R expression and sustained STAT3 activation. Blood 2011; 118:6107-14.

Van Luijn MM, van de Loosdrecht AA, Lampen MH, van Veelen PA, Zevenbergen A, Kester MGD, Falkenburg JHF, de Ru AH, Ossenkoppele GJ, van Hall T, van Ham SM. Promiscuous binding of CLIP to HLA class I unravels invariant chain involvement in the HLA class I antigen presentation pathway of tumors. PLoS One, *in press*.

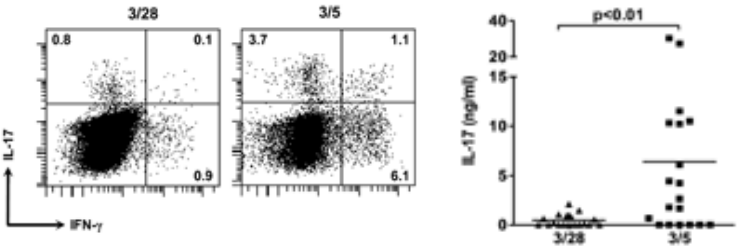

Figure 1: FACS-sorted naive CD4⁺CD45RA⁺CD45RO⁻ T cells were stimulated via coated CD3/CD28 or CD3/CD5 antibodies under Th17 polarizing conditions (IL-23, IL-1β, IL-6, TGF-β and anti-IFN-γ). Left: Intracellular levels of IFN-γ and IL-17A levels were measured at day 11, after 5 hrs of re-stimulation with PMA, ionomycin and BFA. FACS plots shown are from one representative of 18 different donors Right: IFN-γ and IL-17 levels were measured by ELISA at day 11, after 24 hrs of re-stimulation with a CD3-specific Mab (1 µg/ml) and PdBu (50 µM). Data are shown as a mean of 19 individual experiments with different donors.

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Immune modulation by dendritic cells

Immune activation by dendritic cells

In this research line we are developing clinically approved, validated, and cost-efficient monocyte-derived dendritic cell (DC) products. For the development of immuno-activatory DCs we extended our research on our DC maturation-cocktail, monophosphoryl lipid A (MPLA) plus interferon (IFN)γ. We previously demonstrated that these DCs can migrate, produce interleukine (IL)-12 and induce a high percentage of specific and highly cytotoxic T cells (CTLs) against tumor antigens. In addition, the DCs are also able to reactivate these tumor-specific CTLs in blood derived from melanoma patients. These data indicate that the MPLA/IFNγ DCs *in vivo* will preferentially activate inflammatory T cells. Together with the Stem Cell Laboratory, Blood Bank and the Department of Gastroenterology of the AMC we are setting up a phase I/II trial to study the toxicity and use of MPLA plus IFNγ matured DCs in the treatment of patients suffering from esophageal or pancreatic cancer.
In addition, in a more basic research approach we are studying the regulation of the main DC effector functions during maturation. We are exploring the effect of complement activation products, Fc activation and immune complexes on DC function, such as cytokine production, antigen presentation and T cell polarization. In collaboration with Prof Dr Sjaak Neefjes, we studied in a genome-wide siRNA screen approach the regulation of MHC class II antigen presentation in DCs (Figure 2).

Immune tolerance and dendritic cells

Tolerogenic dendritic cells (tDC) are a promising tool as a specific cellular therapy to maintain or restore immunological tolerance in transplantation and auto-immunity. Many described tDC types are not clinically applicable and lack systematic comparison of required functional characteristics, i.e. migratory capacity, stable immunosuppressive phenotype and regulatory T cell (Treg) induction. We previously generated an optimized assay to assess the suppressive capacity of induced Tregs. We have now generated human clinical-grade tDCs using different tolerance-inducing agents. For an optimal migratory and stable phenotype, co-maturation of tDCs with immuno-activatory compounds was required. All tDCs were shown to be highly stable in pro-inflammatory environments, but IL-10 DCs showed the most optimal tolerogenic properties with high IL-10 production, low T cell activation and strong Treg induction. Thus, clinical-grade IL-10 DC show functional characteristics that make them best suited for tolerance-inducing therapies in transplantation or auto-immunity. Furthermore, we study the effect of anti-TNFα biologicals during tDC-T cell co-culture on the T cell polarization and Treg induction.

Key publications:

Paul P, van den Hoorn T, Jongsma M, Bakker M, Hengeveld R, Cresswell P, Egan D, van Ham M, Brinke A, Ovaa H, Beijersbergen R, Kuijl C, Neefjes J. Genome-wide siRNA screen identifies novel pathways controlling MHC class II antigen presentation. Cell 2011; 145:268-83.

Boks MA, Kager-Groenland JR, Haasjes MSP, Zwaginga JJ, van Ham SM, ten Brinke A. IL-10-generated tolerogenic dendritic cells are optimal for functional regulatory T cell induction –- A comparative study of human clinical-applicable DC. Clin Immunol 2012; 142(3):332-42.

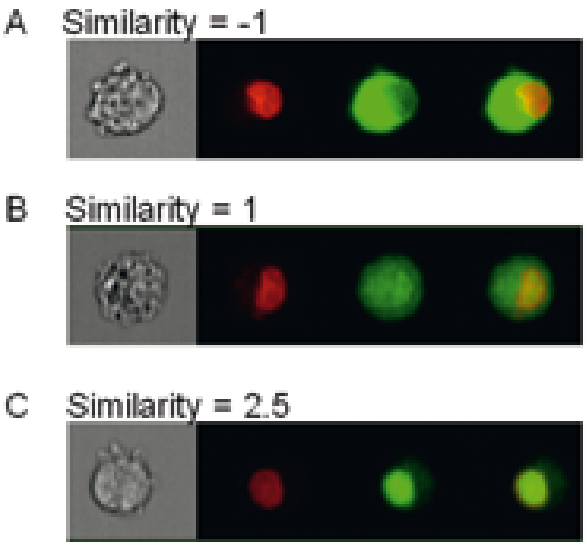

Figure 2: ImagestreamX analysis (Amnis) of NF-κBp65 translocation to the nucleus upon activation of DCs by LPS. Data are depicted as corresponding similarity. Images from left to right: Bright field image, Draq5 nuclear staining (red), fluorescent NF-κBp65 staining (green), merged fluorescent stainings. a) unstimulated, NF-κBp65 is mainly present in the cytosol and not in the nucleus, resulting in a similarity of -1. b) 5 min after LPS stimulation; NF-κBp65 has partly translocated to the nucleus, which resulted in a similarity of 1. c) 30 min after LPS stimulation; NF-κBp65 has fully translocated to the nucleus, corresponding with a similarity of 2.5.

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Human immunoglobulins

Immunoglobulins are key players in immune responses against many pathogens, but also against non-infectious agents such as allergens or therapeutic biomolecules. Depending on the type of antibody, their effects may range from immune activation or target neutralization, to down-modulation of the immune response. It is only partially understood how the many functions of immunoglobulins relate to their structural variability (including subclass, glycosylation profile, and hinge isomers). In a therapeutic setting, antibodies are used to treat a variety of disorders. Stability and composition are major factors influencing efficacy and may lead to adverse effects such as hypersensitivity reactions. For monoclonal antibodies, immunogenicity is increasingly being recognized as a potential threat. Our main theme is to understand the structure-function relationships of different human immunoglobulins. In this context, we focus on three, interconnected topics:

Structural and functional properties of human IgG4

Immunoglobulin (Ig) G4 is a unique antibody with characteristic structural properties that contribute to its status as a 'blocking' antibody. We found that human IgG4 exchanges half-molecules with other IgG4 molecules in blood, yielding antibodies with two different antigen-combining sites. The reaction was not observed upon mixing IgG4 antibodies in buffer, but was seen in both *in vivo* (in a mouse model) and *in vitro* (in the presence of glutathione as catalyst) models. We identified the key structural features that are responsible for this phenomenon using a panel of IgG4/IgG1 mutants in collaboration with Genmab. Also, IgG4 was able to bind to other IgG molecules, in particular, IgG4. This binding is related to the exchange process and may resemble an intermediate step. IgG4 binds to all human IgG subclasses if directly immobilized, but only to IgG4 when bound to antigen. Mechanistic studies are undertaken to establish the individual steps of this process. A FRET assay has been developed to monitor the exchange reaction in real time (Figure 3).

IgG4 stands out also during a T helper cell (Th) 2-driven immune response. Whereas IgG1 antibodies are readily formed, IgG4 antibody titers rise only slowly upon persistent antigenic stimulation. However, the IgG4 response dominates in the end. The underlying mechanisms that control the switch to, and proliferation of, IgG4-producing B cells are only partially understood. We initiated research aimed at unraveling these mechanisms. We quantified the number of IgG4-positive B cells in blood with FACS analysis and *in vitro* culture experiments. In line with the relatively low serum levels of IgG4 (3-4% of total IgG), the number of IgG4$^+$ B cells is correspondingly low. Tools are currently being developed to investigate features that might explain why immune regulation of IgG4 differs from other isotypes.

Key publications:

Labrijn AF, Rispens T, Meesters J, Rose RJ, den Bleker TH, Loverix S, van den Bremer ET, Neijssen J, Vink T, Lasters I, Aalberse RC, Heck AJ, van de Winkel JG, Schuurman J, Parren PW. Species-Specific Determinants in the IgG CH3 Domain Enable Fab-Arm Exchange by Affecting the Noncovalent CH3-CH3 Interaction Strength. J Immunol 2011; 187, 3238-46.

Rispens T, Ooijevaar-de Heer P, Bende O, Aalberse RC. Mechanism of Immunoglobulin G4 Fab-arm Exchange. J Am Chem Soc 2011; 133:10302

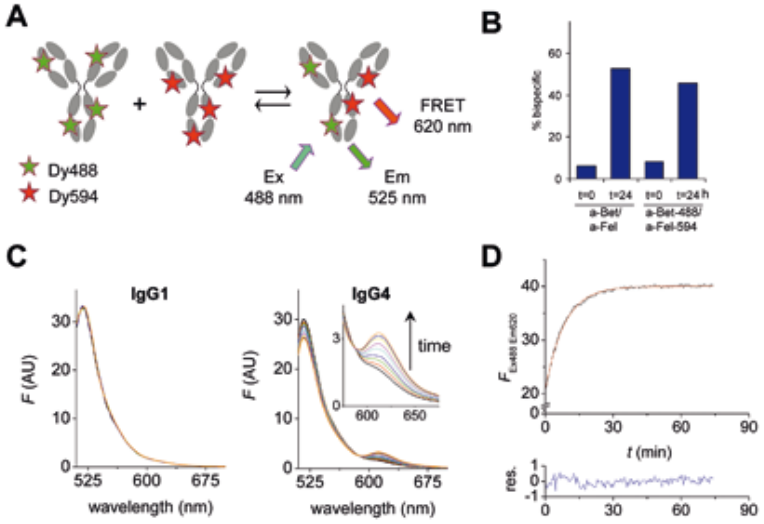

Figure 3: Real-time monitoring of the exchange process using a fluid-phase FRET assay. A) IgG4 is labeled with either Dy488 or Dy594 fluorochrome, and the reaction is monitored by measuring the FRET signal that arises from the mixed exchange product. B) Anti-*Bet v* 1 IgG4 was labeled with Dy488 and anti-*Fel d* 1 with Dy594 and incubated (37°C/1 mM GSH), and in a cross-linking radioimmunoassay using *Bet v* 1 Sepharose and 125I-labeled *Fel d* 1 it was demonstrated that bi-specific antibodies are formed similar to non-labeled antibodies. C) Fluorescence overlay spectra of IgG1-Dy488 and IgG1-Dy594 (adalimumab, left panel) or IgG4-Dy488 and IgG4-Dy594 (natalizumab, right panel) mixed and incubated at 37°C for 60 minutes after reduction with 1 mM DTT. D) Rate profile obtained by monitoring the exchange of DTT-reduced IgG4 by measuring the appearance of a FRET signal at 620 nm. Black curve: experiment; red curve: fit of a first-order exponential. Lower panel shows residuals of fit. (J Am Chem Soc 2011; 133:10302).

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The Department of Immunopathology investigates the regulation of inflammation and tolerance against non-infectious antigens, with a specific focus on humoral immune responses. The research lines can be divided into five main themes. These lines are presented in the PI pages of Lucien Aarden and Marieke van Ham, but are strongly interconnected.

- **The immunoglobulin research group** (Theo Rispens PhD, Gertjan Wolbink MD PhD, Prof Lucien Aarden PhD) aims to understand the structure-function relationships of different human immunoglobulins. Research focuses specifically on the IgG4 B cell response, immune modulation by IVIg (intravenous immunoglobulins) and mechanisms and adverse effects of antibody formation to therapeutic antibodies.
- **The complement research group** (Diana Wouters PhD, Prof Lucien Aarden PhD) is interested in genetic variation and therapeutic application of proteins that regulate complement, as uncontrolled complement activation may lead to inflammatory disease. A new research line focuses on complement-mediated red blood cell destruction as occurring in autoimmune hemolytic anemia.
- **The inflammation research group** (Sacha Zeerleder MD PhD, Prof Lucien Aarden PhD) investigates the role of plasma proteins in inflammation. Protease cascade systems contribute to the removal of 'cellular waste' or induce an inflammatory response upon activation by 'cellular waste'. Current research focuses on the role of Factor VII-activating protease (FSAP) and nucleosomes in inflammatory diseases.

Both research groups collaborate to investigate the function and use of C1-inhibitor, a crucial regulator of the complement and contact system protease cascade systems.

Related research lines presented by Prof Marieke van Ham are:
- Dendritic cells
- B cells

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Human immunoglobulins

Structure-function relationships within intravenous immunoglobulin (IVIg)

Besides being used for replacement therapy in patients with antibody deficiency, intravenous immunoglobulin (IVIg) is used in conditions such as Idiopathic Thrombocytopenia Purpura (ITP), Kawasaki syndrome and Guillain-Barre. In applications other than replacement therapy, the mechanisms of action are largely uncertain, and proposed mechanisms are for example, effects due to scavenging of complement activation products, blockade of Fc receptors, effects of IgG dimers and effects of specific antibodies (for example: cytokine neutralization).

We investigated properties such as stability of the IgG dimers present in IVIg under different physical conditions by among others size-exclusion chromatography and sodium dodecyl sulfate (SDS) electrophoresis. A substantial fraction of dimers dissociate rapidly under conditions mimicking those in patients after administering IVIg, but part of the dimers remain stable. Formation of dimers and larger aggregates may result in part from slightly denatured IgG. We found that aggregation of IgG may be counteracted by addition of fragments of IgG.

Treatment of conditions such as ITP require high doses of IVIg. It is reported that only the fraction of IgG molecules containing sialic acid is responsible for its anti-inflammatory action. These findings are based mainly on an arthritis mouse model. In cooperation with the Division of Plasma Products, IVIg was enriched for sialic acid (SA). The SA-enriched and depleted IVIg were tested in a mouse model for ITP.

Monomeric precursors for aggregation of IgG are also difficult to detect. Usually, hydrophobic fluorescent probes such as 1-anilino-8-naphthalenesulfonate are used that may detect exposed hydrophobic surfaces as a result of partial unfolding. We extended this approach by detecting binding of such probes using isothermal titration calorimetry. We also developed a method of detecting small quantities of alternatively-folded IgG using radio-labeled IgG fragments.

Key Publications

Guhr T, Derksen N, Aalberse R, Rispens T. Use of a Human Recombinant Immunoglobulin G1 CH3 Domain as a Probe for Detecting Alternatively Folded Human IgG in Intravenous Ig Products. J Pharm Sci 2012; 101(3):978-86.

Guhr T, Bloem J, Derksen NI, Wuhrer M, Koenderman AH, Aalberse RC, Rispens T. Enrichment of sialylated IgG by lectin fractionation does not enhance the efficacy of immunoglobulin G in a murine model of immune thrombocytopenia. PLoS One 2011; 6(6):e21246

Hawe A, Rispens T, Herron JN, Jiskoot W. Probing bis-ANS Binding Sites of Different Affinity on Aggregated IgG by Steady-State Fluorescence, Time-Resolved Fluorescence and Isothermal Titration Calorimetry. J Pharm Sci 2010 Oct 18 [Epub ahead of print]

Immunogenicity of biologicals

Increasing numbers of proteins are used as therapeutic agents. In particular, therapeutic monoclonal antibodies are used increasingly to treat a variety of disorders such as rheumatoid arthritis. Therapeutic proteins can induce an unwanted antibody response that diminishes treatment efficacy. Even fully human therapeutic antibodies can induce an antibody response. We study the responses to various therapeutic antibodies (anti-idiotype, neutralizing/non-neutralizing), the biology of immune complexes, and the regulation of B cell responses to therapeutic antibodies. This project is also firmly linked to Sanquin Diagnostic Services. Assay development is an integral part of this project resulting in new tests for immunogenicity testing.
Even where the therapeutic mAb is fully human, anti-idiotypic responses are elicited in a considerable number of the patients. This leads to reduced efficacy due to blocking of the therapeutic drug by anti-drug antibodies. Patients may also develop treatment-related side effects due to the formation of immune complexes (IC). We studied antibody formation in rheumatoid arthritis patients treated at 2-weekly intervals with the fully human anti-TNFα antibody adalimumab and found that within 6 months of treatment 70% of the patients produced anti-adalimumab antibodies, mostly of the IgG1 and IgG4 subclass. Small circulating IC could be detected in most patients even two weeks after the administration of adalimumab. In other words, these patients are permanently exposed to high concentrations of small IC.

Key Publications

Jamnitski A, Krieckaert CL, Nurmohamed MT, Hart MH, Dijkmans BA, Aarden L, Voskuyl AE, Wolbink GJ. Patients non-responding to etanercept obtain lower etanercept concentrations compared with responding patients. Ann Rheum Dis 2012; 71:88-91.

Hart MH, de Vrieze H, Wouters D, Wolbink GJ, Killestein J, de Groot ER, Aarden LA, Rispens T. Differential effect of drug interference in immunogenicity assays. J Immunol Methods 2011; 372:196-203.

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Jamnitski A, Bartelds GM, Nurmohamed MT, van Schouwenburg PA, van Schaardenburg D, Stapel SO, Dijkmans BA, Aarden L, Wolbink GJ. The presence or absence of antibodies to infliximab or adalimumab determines the outcome of switching to etanercept. Ann Rheum Dis 2011; 70:284-8.

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Complement

Complement is part of the innate immune response and very powerful inflammatory reactions are induced upon activation of the complement system. Uncontrolled complement activation by either excessive activation or lack of regulation may lead to inflammatory disease. We are interested in the genetic variation and therapeutic application of proteins that regulate complement. In cooperation with Prof Taco Kuijpers (Academic Medical Center) we study the molecular organization of the lectin pathway and investigate why complement activation is attenuated in pediatric oncology patients. More recently we started a new research line in which we study complement-mediated red blood cell destruction as occurring in autoimmune hemolytic anemia. Both *in vitro* lysis of red blood cells and C3 deposition on the red blood cell surface could be inhibited by a high dose of C1-inhibitor. The therapeutic monoclonal anti-C5 antibody only inhibited lysis, while leaving C3 deposition on the surface intact.

C1-inhibitor

C1-inhibitor (C1-inh) is a major Sanquin therapeutic product. We compare properties of recombinant C1-inh with plasma-derived C1-inh and investigate the effects of (glycosylated) C1-inh on neutrophil/endothelial cell interaction. In collaboration with Sanquin Plasma Products and Viropharma we are exploring possible new fields of clinical application for C1-Inh. In collaboration with Prof Hans Niessen (Departmentt of Pathology, VUmc) we demonstrated in a rat burn wound model that systemic treatment with C1-inh improves the local healing of burn wounds and reduces inflammation in the heart. We will further explore the possibility of C1-inh as therapeutic intervention for burn wound patients. We also showed in an *ex vivo* model for acute vein graft injury that C1-inh protects against endothelial loss under arterial blood pressure.

Key publications:

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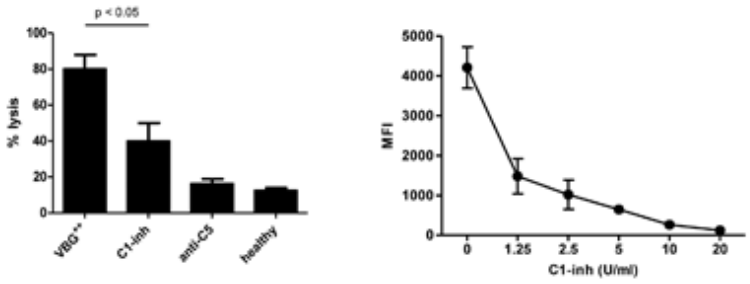

C1-INH inhibits hemolysis induced by AIHA patient serum
A. Hemolysis of bromelain treated human O-typed erythrocytes upon incubation with AIHA patient serum is inhibited by C1-INH (20 U/ml) and mAb anti-C5 (100 µg/ml). Mean and SEM (error bars) are shown of six AIHA patients.
B. Dose-dependent inhibition of C3 deposition on RBC surface by C1-inh.

Inflammation

Clearance of dead cells is facilitated by a large number of plasma proteins. We study the role of Factor-VII-activating protein (FSAP) in the clearance of dead cells. FSAP circulates in plasma as an inactive single chain serine protease. FSAP binds to living as well as to dead cells. Interaction with dead cells leads to the activation of FSAP and to the release of nucleosomes. Although we do not know how FSAP is activated, exposure to nuclear proteins such as histones seems to be essential for this activation. Cell death is a central event in the pathogenesis of sepsis and is reflected by circulating nucleosomes. Circulating nucleosome levels correlate with severity and outcome in sepsis patients. Therefore we investigated FSAP activation in patients suffering from various inflammatory diseases of increasing severity. We developed ELISAs to measure FSAP-C1-inhibitor and FSAP-α2-antiplasmin complexes in plasma. FSAP-inhibitor complexes were measured in the plasma of 20 adult patients undergoing transhiatal esophagectomy, 32 adult patients suffering from severe sepsis, 8 adult patients suffering from septic shock and 38 children suffering from meningococcal sepsis. FSAP activation occurred in post-surgery patients, patients suffering from severe sepsis, septic shock and meningococcal sepsis. Levels of FSAP-inhibitor complexes correlated with nucleosome levels and correlated with severity and mortality in these patients. These results suggest that FSAP is a sensor for cell death and that FSAP activation in sepsis might be involved in nucleosome release, thereby contributing to lethality.

Key publications:

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Blood-borne Infections

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In 2011 the 'Blood-borne Infections' Department (BOI) consisted of the following permanent staff: one full-time technician, two molecular biologists, each working 0.5 FTE; and one manager/microbiologist, working 0.7 FTE. In addition, one senior researcher/risk specialist has been appointed for the duration of a two-year project. BOI has two PhD students, working on 'The HBV X protein' and 'Emerging infections in the Netherlands', respectively

Research lines

- The policy for emerging infections
- Endemic hepatitis E
- Molecular epidemiology of parvovirus

The policy for emerging infections: do we take safety measures or not?

Originally, the researcher engaged for two years started on the risk inventories and analyses to support the project 'Fast and standardized decision-making in case of emerging infections´, as planned. A crucial point of the policy in the field of blood safety, particularly concerning emerging infections, though, was found to be that we lack a basic, conscious policy for handling new, small risks and new, unknown risks to blood safety. At first glance, it seems that from the viewpoint of 'maximal safety', measures should be taken against every threat. In practice, however, measures against infectious threats have varying results. Classic, rare infections that have been emerging for decades are sometimes accepted without preventive measures, while new outbreaks that catch people's attention generally lead to preventative measures. Apparently, numerical risk reduction is only one of the driving factors behind the decision whether or not to institute measures. This phenomenon is easily understood, but it reduces the usefulness of the purely medical-economic (cost/benefit) approach used so far to answer the question of whether an outbreak deserves prevention. Due to this growing insight, the researcher focused on the analysis of a situation with tough preventative measures for a small risk (Prinsze et al., 2012). Subsequently, a more basic analysis was conducted of the usefulness of the many targeted measures for local outbreaks elsewhere, leading to (submitted): "The yield of temporary exclusion of traveling blood donors", by Prinsze et al.

Key publication

Prinsze FJ, Zaaijer HL. The outcome of donor screening for Human T-Cell Lymphotropic Virus infection in the Netherlands. Vox Sang 2012; 102(3):198-203.

Endemic hepatitis E

Recently, it was found that dozens of Dutch patients undergoing immune suppression subsequent to transplantation or hematological conditions are chronically infected with hepatitis E virus (HEV). These patients have been exposed to blood products, which poses the question of whether donated blood could be a source of HEV infection. During the course of 2011 BOI initiated a major study into the occurrence of HEV infection in blood donors. On two work days in 2011, every donor was asked to cooperate in a study on the occurrence of (silent) infection with HEV. Three assays for the detection of antibodies to HEV were compared, and a PCR for the detection of HEV RNA was developed. During the course of 2012 the results of this study will be ready.

Molecular epidemiology of parvovirus

Every year there is a seasonal rise in silent infections with parvovirus-B19 (B19V) in our blood donors, with a pronounced increase about every fourth year. The level of viraemia in the otherwise symptom-free donors can be very high. Given the risk of this phenomenon, 'parvo-safe' blood has been made available in the Netherlands for vulnerable patients. To gain some insight into the epidemiology of the silent B19V outbreaks in asymptomatic blood donors, the genome of isolates from viraemic donors was mapped using sequencing. Then the B19V isolates were compared to each other and finally compared to B19V isolates from the RIVM, obtained from children with symptomatic B19V infection (the 'fifth childhood disease').
There appear to be B19V infections with genotype 1a, with no genetic drift occurring in time or place; there is also no difference between donor and clinical B19V isolates. A new, basal-virological phenomenon was revealed. It seems that, around the world, the genomes of B19V-1a can be divided into 2 subtypes, based on 2 sets of synonymous mutations spread throughout the entire genome. The biological significance of this finding is unknown.

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Transfusion Technology Assessment

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The Transfusion Technology Assessment (TTA) group, established in 2004, is an ongoing Sanquin Blood Supply Foundation collaboration with the Medical Technology Assessment department of the Julius Center for Health Sciences and Primary Care at Utrecht University. The group was installed to perform risk assessment and economic evaluations on (interventions targeted at) blood safety issues. Since its conception, TTA has also established models for clinical blood use, blood recipient profiles and collected and analyzed data on European blood use and supply. The TTA group provides scientific publications and also proprietary information to the Sanquin Executive Board and the Plasma Products division. In recent years, in the course of the PROTON study, the TTA group has compiled an invaluable source of information on the blood transfusion chain that is unique in the Netherlands and rare in the world.
The TTA group has identified four main research themes: optimal blood safety, optimal blood use, optimal blood supply and methodology development.

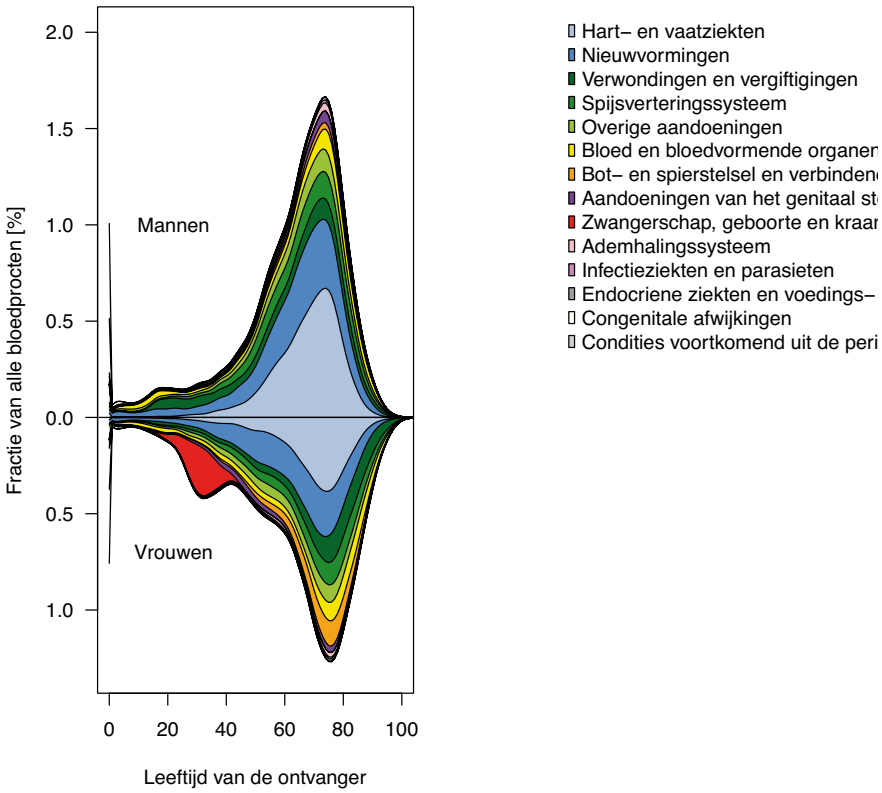
In 2011 the TTA group received two awards:
- The Best Paper Prize for the best original article published in Vox Sanguinis in 2010 was awarded to the paper by Borkent-Raven BA, Janssen MP, van der Poel CL, Schaasberg WP, Bonsel GJ, van Hout BA: The PROTON study: profiles of blood product transfusion recipients in the Netherlands. Vox Sang 2010; 99(1):54-64.
- The Risk Management Study Award from the Dutch Risk Management Society (GvRM) and the Dutch Risk and Reliability Association (NVRB) was awarded to MP Janssen for his PhD Thesis 'Modeling Blood Safety', ISBN 978-90-393-5401-8.

We are currently focusing on two main projects, but there are also some other ongoing activities:
- PROTON2 study
- MITCH study
- Other activities

Profiles of Transfusion Recipients (PROTON2-study)

Data on blood recipients from 1995 – 2006 was collected in 20 Dutch hospitals to establish the PROTON dataset, containing 2.4 million transfusions and their recipients. Data were linked to mortality databases (GBA) and hospital discharge diagnoses databases (LMR) at Statistics Netherlands (CBS). The PROTON dataset encompasses 28% of the total blood use in the Netherlands. In collaboration with Clinical Consultancy Services of the Sanquin Blood Bank and the AIM tool developed by American Blood Centers, TTA has started a program of Data ANalysis for ongoing OPtimization of the TRAnsfusion chain (DANOPTRA) in 2011, which is referred to as PROTON2. This project was initiated with the vision of developing an infrastructure for ongoing modeling studies. Additional collaboration with the EMGO+ institute (Amsterdam) and the Jon J van Rood Center for Clinical Transfusion Research (Leiden) will lead over the next few years to the implementation of a system of continuous collection of data on the Dutch blood transfusion chain. Data will be collected in one central data warehouse to allow for answering a range of research questions for various stakeholders. The PROTON2 dataset will allow:

- (Inter)national benchmarking of hospitals on blood use characteristics (e.g. blood use per patient subgroup, outdating, shelf life, transfusion triggers). This can support managerial and/or logistic optimization and the development of guidelines on optimal blood use.
- Trend analysis on clinical blood use among different patient subgroups and age distributions as well as changing indications and transfusion triggers which can be used to estimate future blood demand (optimal blood supply).
- Quantitative analyses of (adverse) clinical outcomes and transfusion recipient survival in relation to various determinants in donor, product and/or production and recipient characteristics (optimal blood safety).



Distribution of transfused blood components by age, gender and patient discharge diagnosis

Key Publications

Borkent-Raven BA, Janssen MP, van der Poel CL, Schaasberg WP, Bonsel GJ, van Hout BA. The PROTON study: profiles of blood product transfusion recipients in the Netherlands. Vox Sang 2010; 99(1):54-64.

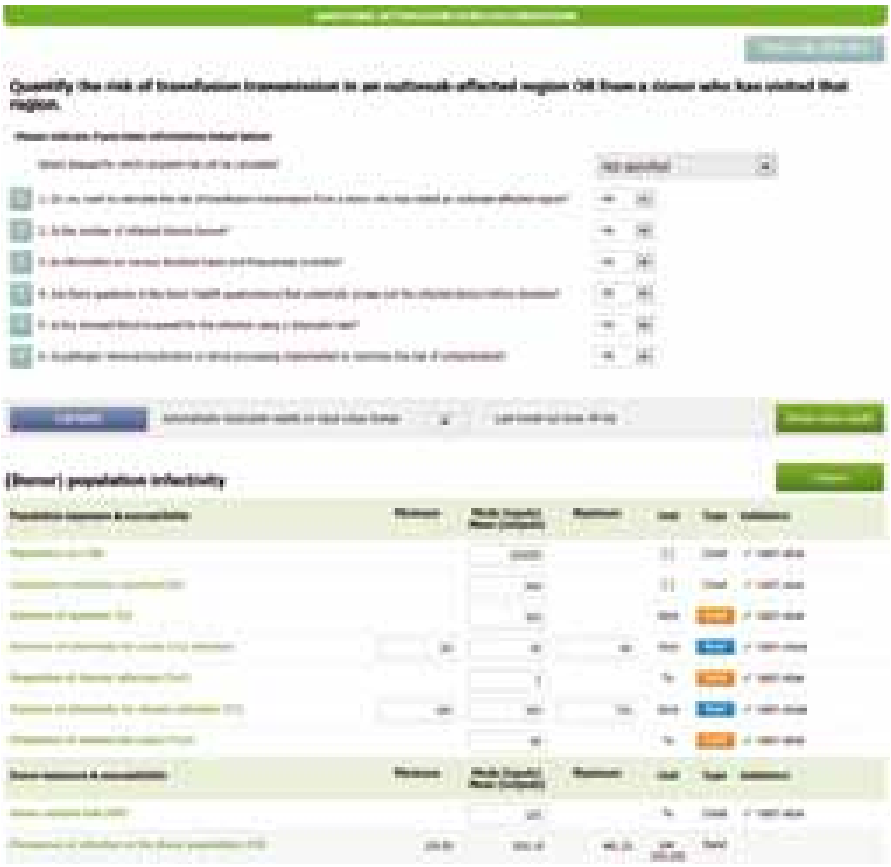
Borkent-Raven BA, Janssen MP, van der Poel CL, Schaasberg WP, Bonsel GJ, van Hout BA. Survival after transfusion in the Netherlands. Vox Sang 2011; 100(2):196-203.

Borkent-Raven BA, Janssen MP, van der Poel CL. Demographic changes and predicting blood supply and demand in the Netherlands. Transfusion 2010; 50(11):2455-60.

Borkent-Raven BA. The PROTON study: Profiles of transfusion recipients in the Netherlands (PhD Thesis), 2010 Utrecht University, The Netherlands: ISBN 978-90-393-5407-0.

Modeling Infections in the Transfusion Chain (MITCH-study)

The Utrecht Center for Infection Dynamics (UCID) performs mathematical modeling on infectious disease epidemiology, and is a collaboration between the Center for Infectious Disease Control (RIVM/ CIb) and the Julius Center. TTA collaboration with UCID strengthens the knowledge base for blood safety modeling. Generic Modeling of Infections in the Transfusion Chain (MITCH) is in development, encompassing all relevant parameters for biological and epidemiological characteristics of infections in the transfusion chain. MITCH will allow the ad hoc introduction of actual EID parameters, yielding timely assessment for risk management. In collaboration with UCID and the Sanquin Department of Blood-borne Infections, modeling of Q-fever risk during outbreaks in the Netherlands was started. Given recent Q-fever, Chikungunya and Dengue outbreaks in Europe, the European Centers of Disease Control (ECDC) asked TTA to develop a European Up-Front blood Risk Assessment Tool (EUFRAT), a flexible web-based EID modeling tool to provide quick access by EU experts and decision makers. In the course of 2011 this tool was successfully finalized, presented and tested at various meetings. Further development and dissemination of the tool is currently under ECDC consideration.



Screenshot of the EUFRAT web-based risk assessment tool

Other activities

In addition to the main research projects the TTA group also performed a number of smaller projects:

- Annual reporting on the collection, testing and use of blood components in Europe has been assigned to the TTA group since 2001 by the Council of Europe. The data collected for reporting year 2009 were analyzed, reported and also an update of the earlier trend analyses, now including reporting years 2001 through 2008, was performed and reported. The final reports will be published by the Council of Europe, EDQM Department of Biological Standardization, OMCL Network and Healthcare, Strasbourg.
- As a result of the work done for the Council of Europe a paper was written on the trend analysis methodology applied. This work is currently under review.
- The TTA group was asked to perform trend analyses for the WHO on the Global database on blood safety.

Council of Europe 2008 Report on the Collection, Testing and Use of Blood and Blood Components in Europe

Key Publications

Van der Poel CL, Janssen MP, Behr Gross ME. The Collection, Testing and Use of Blood and Blood Components in Europe, 2008 Report. Department of Biological Standardization, OMCL Network & HealthCare, European Directorate for the Quality of Medicines and Healthcare (EDQM), Council of Europe, May 2011.

Van der Poel CL, Janssen MP, Behr Gross ME.Trends and observations on the collection, Testing and use of blood and blood components in Europe, 2001-2005 report. Department of Biological Standardization, OMCL Network & HealthCare, European Directorate for the Quality of Medicines and Healthcare (EDQM), Council of Europe, November 2010.

Transfusion Technology Assessment

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Transfusion Monitoring

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The Department of Transfusion Monitoring (TraM) in Groningen is involved in many fields of Blood Banking, such as transfusion monitoring (e.g. BCSI large field study), optimization of quality and processing of blood products (e.g. the stem cell freezing process, proficiency studies), contract research (e.g. PRA project), cryolijm-SQ, and studies in cooperation with the UMCG.

Our three main research lines are:

- Improvement of storage of blood products
- Development of new products
- Transfusion monitoring

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Improvement of storage of blood products

Stem cell studies

The Department of Transfusion Monitoring performed various research projects for the stem cell department to optimize the freezing process for stem cells. Because hematopoietic progenitor cells (HPC) are not readily available for research purposes, an HPC simulant was developed from buffy coats and plasma, with comparable cell counts and hematocrit but the absence of $CD34^+$ cells. An infusion pump for DMSO was tested to add DMSO in a more controlled way (Figure 1). It appeared that the accuracy of the added volume was too large and therefore the manual addition of DMSO is still in use. The concentration of DMSO that is needed to be added before freezing was studied. Results indicated that viability of WBC is higher when 20% DMSO is used compared to when 50% DMSO is used as initial DMSO concentration to add to the HPC simulant. Final volume and WBC count of the product were not of influence. Reduction of the volume by centrifugation might lead to a lower viability. Various freezing bags have been tested and one of these has been validated for routine use, because the standard freezing bag will no longer have the requested CE mark. Since the large molecular weight (200 kDa) hydroxyethyl starch (HES) will no longer be available for sedimentation of bone marrow stem cells, comparison experiments were done with lower molecular weight (130 kDa) HES. Sedimentation without HES was also tested. The RBC depletion was highest for HES of 200 kDa and for no HES, while WBC recovery was acceptable in all tested conditions. Besides this, tests for measuring vitality after thawing have been validated. It appears to be important to use the right thawing solution and cell count.

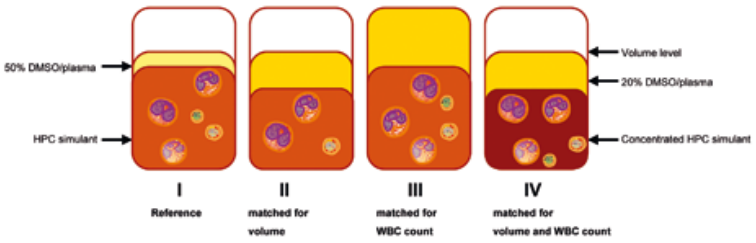

Figure 1: The concentration of DMSO that is needed to be added before freezing is studied using four study conditions.

Key publication

Dijkstra-Tiekstra MJ, Setroikromo AC, de Wildt-Eggen J. Freezing 'stem cells' in a bag and tube under various freezing conditions? Vox Sang 2012; 102(3):273

Proficiency studies

Every year TraM organizes proficiency studies to compare the results of different instruments used within the various departments of Sanquin Blood Bank. Samples are prepared centrally at TraM and distributed to the participants. Afterwards the results are collected and analyzed. Reports with conclusions are written and sent to the different departments. In 2011 five national proficiency studies were executed.
(I) Comparison of cell counters for counting platelets in platelet concentrates and storage solution
(II) Comparison of flowcytometric instruments for counting leukocytes in plasma, red cell concentrates, platelet concentrates and platelet storage solution
(III) Comparison of blood gas analyzers for measuring pH in platelet concentrates
(IV) Comparison of flowcytometric instruments for measuring platelet activation in platelet concentrates using CD62p expression and Annexin V binding
(V) Comparison of flowcytometric instruments for counting platelets using CD41 expression.
For the proficiency study I and V trend analysis reports were also written, using data from 2006 to 2011.

Key publication

Van der Meer PF, Karssing-van Leeuwen W, Kurtz J, Spengler HP, Blair A, Devine D, Harrison P, Lambrecht B, Vandenbroeke T, de Wildt-Eggen J, de Korte D; on behalf of the Biomedical Excellence for Safer Transfusion (BEST) Collaborative. A flow cytometric method for platelet counting in platelet concentrates. Transfusion 2012; 52:173-80.

Contract research

This year TraM performed contract research for PRA International. During five months almost 500 samples were tested for CD34 amount. Before tests could be started an additional validation was required in accordance with the specifications of PRA International.

Development of new products

Cryolijm-SQ studies

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From 2008 cryolijm-SQ, a fibrin glue of single donor plasma which Sanquin has started to produce as an alternative for autologous or pooled plasma fibrin glues, is produced in a routine setting. In 2009 TraM started a formal stability study to officially confirm the two-year storage life of cryo-SQ. The formal stability study was completed in 2011; all tested samples met the predetermined requirements.
In 2011 the shelf life of two years was processed in eProgesa.
The integrity of the over-wrap of the syringes containing cryolijm-SQ was also tested.
To be able to produce autologous cryolijm and because of the interest in cryolijm-SQ from foreign countries, the production of cryolijm-SQ from whole blood-derived plasma and fresh apheresis plasma was validated and approved.
Austrian whole blood-derived inline filtered plasma units could be used for the production of cryolijm-SQ for the European market. The validation was initiated in Q4 2011.
Sanquin is also creating the possibility of transferring the technology of cryolijm-SQ production to foreign countries. A start was made in creating a training program.

Other studies in correlation to cryolijm-SQ are:

FIRST-study: The first inclusion was in January 2011 and the study will be completed in 2012.
Freezing curve: The freezing and thawing curve of cryolijm were determined. Packed in a plastic bag it can be left at room temperature for a maximum of 3:45 minutes when taken from the -30°C and 4:30 minutes when packed in a cardboard box before reaching a temperature of -18°C.
Anti-AB titer: Cryolijm-SQ can be used irrespective of blood group. Since the anti-A and anti-B titers of cryolijm-SQ produced from blood group B and A plasma units respectively were not known, these were determined in cryoprecipitate. The obtained titers in the cryoprecipitate from blood group A plasma units as well as blood group B plasma units were within the requirements of the Blood Products Guideline for anti-A and/or anti-B titers for children's platelet concentrates.
Extending clotting time: Cryolijm-SQ is particularly suitable in achieving haemostasis. When used as an adhesive, in some cases a longer clotting time would be preferable. With pilot experiments it was shown that it is possible to extend the clotting time by diluting the thrombin component of cryolijm-SQ. This research will continue in 2012.
Use of cryolijm-SG for upper eyelid blepharoplasty: Together with the UMCG, Department of Plastic Surgery, a research proposal was submitted for a study to investigate whether cryolijm-SQ can be used instead of sutures, so that the hospital visit to remove the sutures is not necessary. This study will start in 2012.

Key publication

Hazelaar S, Dijkstra-Tiekstra MJ, de Korte D, de Wildt-Eggen J. Allogeneic single-donor cryoseal produced from fresh-frozen quarantine aphaeresis plasma as alternative for multidonor or autologous fibrin sealants. Transfusion 2012; 52(3):517-23.

BCSI studies

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In 2009 and 2010 TraM performed a large field evaluation involving five blood bank departments and four participating hospitals. Blood Cell Storage, Inc. (BCSI) has developed a detection system to measure the pH in platelet concentrates (PCs) in a non-invasive way.
In 2011 the data were analyzed further. In the study very few PCs with low pH were found. A low pH seemed to be correlated to a positive BacT/Alert or a high platelet count. No correlation was found between pH of the PC and the corrected count increment (CCI) of the patient (Figure 2). A pH <7.1 on day 7 seemed to predict a high annexin V binding on day 9.
Additionally, the BCSI pH meter was studied to measure pH in PCs made from colored plasma and for PCs in synthetic additive solutions. For PCs in brown, fat, green, red or normal colored plasma an increased deviation was seen in pH measured on BCSI pH1000 compared to the blood gas analyzer. Only PCs in 100% PAS II showed an acceptable deviation. It is expected that the fact that the pH was measured in an optical way using a fluorescent dye plays a role in this. For PCs in various synthetic additive solutions it appeared that the pH deviation compared to the pH as measured using the blood gas analyzer depends on the kind of solution used. However, the deviation remained quite stable during storage. Calibration for each additive solution is therefore recommended.

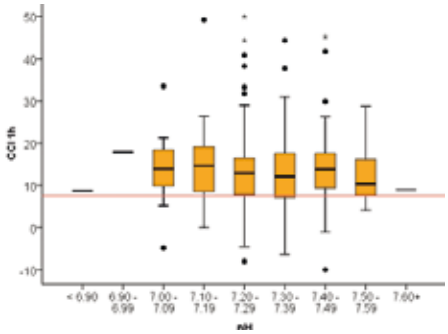

Figure 2: In the BCSI large field study no correlation was found between the pH of the PC and the CCI of the patient. Boxplot formed by median, first and third quartile and most distant observation within 1.5x box, • outlier, * extreme outlier > 3x box

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Transfusion Monitoring

Clinical project: Enhancing quality of Autologous Stem cell products in cooperation with UMCG's Department of Hematology

This project aims at enhancing the quality and quantity of stem cells in autologous transplants and was executed with financial support from Tekke Huizinga Fonds. Over the years Sanquin performed stem cell collection and processed the autologous products, executed in close cooperation with the UMCG which treats and transplants the patients.

Therapeutic Apheresis

Sanquin performs therapeutic plasma apheresis and stem cell collection in a number of hospitals. A large number are done for the Department of Nephrology of the UMCG. To add to improvement of performance and define indications, the method used to estimate the plasma volume was evaluated, as was the indication for plasma apheresis in atypical HUS.

Key publications

Woolthuis C, Agool A, Olthof S, Slart RH, Huls G, Smid WM, Schuringa JJ, Vellenga E. Auto-SCT induces a phenotypic shift from CMP to GMP progenitors, reduces clonogenic potential and enhances in vitro and in vivo cycling activity defined by (18)F-FLT PET scanning. Bone Marrow Transplant 2011; 46:110-5.

Van der Wijk J, Smid WM, Seelen MA, van de Kar NC, Offerman JJ, Son WJ. Renal transplantation in patients with atypical Hemolytic Uremic Syndrome. Neth J Med 2011; 69:279-80.

Transfusion Monitoring

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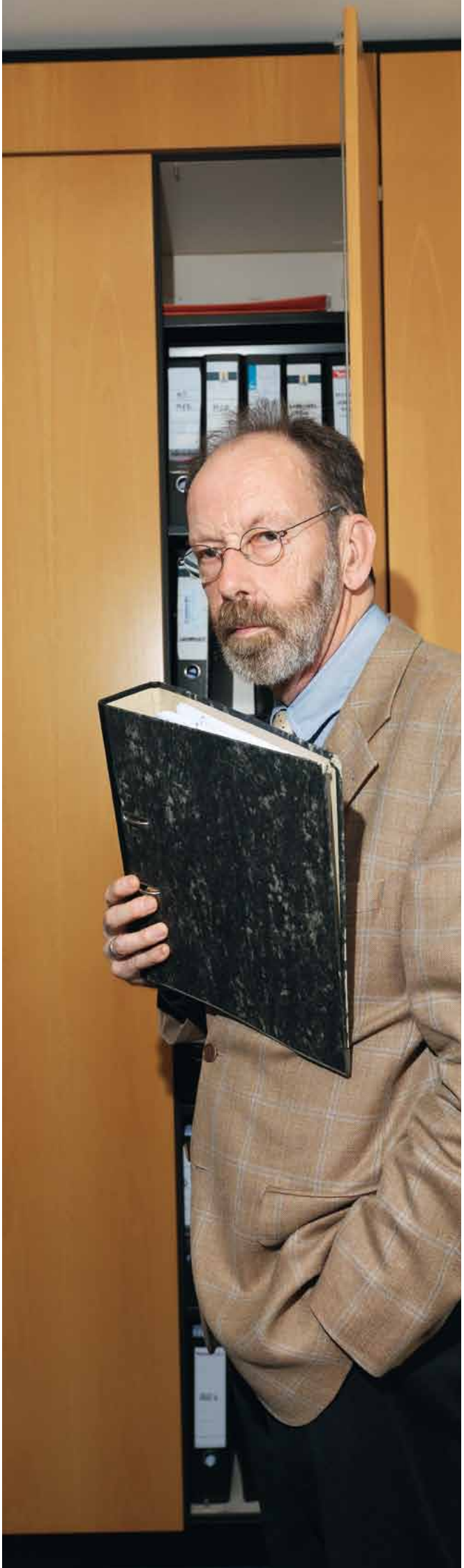

Transfusion Medicine

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In 2010 the Jon J van Rood Center was established to organize and optimize the patient-oriented transfusion research in the Netherlands. Patient-oriented transfusion research involves a multitude of professionals from donor to patient. Our research is therefore performed in close collaboration with professionals from other departments within Sanquin, and from the academic and non-academic hospitals in the Netherlands and abroad.
We focus on patient-oriented research within our main research lines, but the Van Rood Center also offers assistance to transfusion research study design, database development and data management, statistical analyses, and laboratory facilities enabling fast, point of care assays such as on hemostatic capacity, or cellular markers for tracing purposes. The center has special expertise in the implementation of new and proper epidemiological methods which are crucial for high-quality research in this area. We also organize special educational activities such as our renowned weekly national inspirational meeting (Friday lessons), and the two-yearly Boerhaave Course on Advances in Transfusion Medicine.
Our ultimate goal is to improve patient care, as our mission statement indicates:

The Jon J van Rood Center for Clinical Transfusion Research is committed to producing and distributing the best-quality knowledge on transfusion medicine and by doing so to optimize the care of patients who need blood products.

Main Research Lines

- Immunology of blood transfusion
- Hemostasis
- Cell Therapy
- Blood management and side-effects

Immunology of blood transfusion

Our immunology research builds on a long-standing collaboration with the Department of Immunohematology of the Leiden University Medical Center. It focuses on the etiology and mechanisms of immune responses to blood transfusion, as well as on the consequences of these responses for prognosis and management. Both newly-collected data and data from databanks and biobanks are used. In 2011 we worked on the following projects:

LOTUS study

The LOTUS study was set up to describe the incidence and long-term persistence of fetal versus (intra-uterine) transfusion-induced antigen exposure and its relationship with HLA-antigens, HLA-antibodies and fetal chimerism in a large cohort of women treated for hemolytic disease of the newborn between 1987 and 2009. Outcomes are immunologic (high respondership, antibody persistence, chimerism) and child developmental parameters. In 2011 two addendums were METC-approved, mothers and their children who were treated with intra-uterine transfusion (IUT) for non-RBC alloimmune reasons (e.g. Parvo, fetomaternal hemorrhage and non-immune fetal hydrops) were added in accordance with study protocol and the maternal grandmothers of the IUT children to study a NIMA effect on alloimmunization. A total of 263 mothers and their children participated in the LOTUS study, 54 families for addendum-1 and 137 grandmothers for addendum-2.

MATCH study

The MATCH study is a randomized controlled trial that assesses the cost-effectiveness of pre-emptive Rh, K, Fy-a, M, and S antigen matching of red blood cells transfusions. Potential transfusion patients with random and high alloimmunization risk were stratified and randomized to receive routine versus extended (RH, K, Fya, Jka and S) matched red cell transfusion. Included patients were followed over time for red blood cell immunization. By the end of 2011, 846 patients had been randomized, 322 received transfusion and follow-up had been (partly) completed for 205 patients in the four hospitals that participated.

Intra-uterine transfusions

This study explored the use of a single donor for the total intra-uterine transfusion (IUT) treatment period. IUT treatment for fetuses with alloimmune hemolytic disease comprises three transfusions (median; range 1-8) of 10 ml to 150 ml, depending on gestational age administered for a six-week period (median; range 2-20 weeks). For blood donation of small volumes the anti-coagulant volume was adjusted to the donation volume to maintain the standard ratio. After leukocyte-depletion the red blood cells can be stored for a maximum of 72 hours without negative effects on metabolic parameters. In a closed system (Sepax, BioSafe) we developed a leukocyte-depleted red blood cell product with reduced anti-A and anti-B titers from smaller blood volumes (< 100 ml) of volunteer donors with the required hematocrit of 0.8-0.85 l/l. In a previous project for autologous transfusion in premature neonates we showed that it is possible to make RBC products from volumes below 20 ml. The process will be optimized in collaboration with the Sanquin Blood Bank Product and Process Development Department. The next step is to develop the logistic steps from donor to the clinic in collaboration with the Department of Donor Studies. The knowledge from this study may open the opportunity for the preparation of other small volume (neonatal) transfusions.

R-FACT study

The R-FACT study is a nationwide case-control study into the association between clinical, environmental and genetic risk factors of red blood cell alloimmunization. The backbone of this study is a unique cluster of observational (both laboratory as well as clinical) transfusion-related databases between hospitals.

Key publications

Verduin EP, Lindenburg IT, Smits-Wintjens VE, van Klink JM, Schonewille H, van Kamp IL, Oepkes D, Walther FJ, Kanhai HH, Doxiadis II, Lopriore E, Brand A. Long-Term follow up after intra-Uterine transfusionS; the LOTUS study. BMC Pregnancy Childbirth 2010; 10:77.

Eckhardt CL, van der Bom JG, van der Naald M, Peters M, Kamphuisen PW, Fijnvandraat K. Surgery and Inhibitor Development in Hemophilia A: a Systematic Review. J Thromb Haemost 2011; 9(10):1948-58.

Van de Stadt LA, de Koning MH, van de Stadt RJ, Wolbink G, Dijkmans BA, Hamann D, et al. Development of the anti citrullinated peptide antibody repertoire prior to the onset of rheumatoid arthritis. Arthritis Rheum 2011; 63(11):3226-33.

Zalpuri S, Zwaginga JJ, le Cessie S, Elshuis J, Schonewille H, van der Bom JG. Red-blood-cell alloimmunization and number of red-blood-cell transfusions. Vox Sang 2012; 102(2):144-9.

Natukunda B, Mugyenyi G, Brand A, Schonewille H. Maternal red blood cell alloimmunisation in South Western Uganda. Transfus Med 2011; 21(4):262-6.

Hemostasis

Our research on hemostasis addresses treatment as well as prophylaxis of bleeding in various kinds of clinical situations. Ongoing projects include women with perinatal bleeding and patients with chemotherapy-induced thrombocytopenia.

WOMB study

In 2011 we finished the WOMB study and the main findings are being analyzed. The study was a nationwide multicenter randomized trial in patients with post-partum hemorrhage, where a restrictive red blood cell transfusion policy was compared with a more liberal red blood cell transfusion policy. Women suffering from post-partum hemorrhage (e.g. blood loss ≥1000 ml or hemoglobin (Hb) decrease ≥1.9 g/dl) resulting in Hb values between 4.8 and 7.9 gr/dl, without physical complaints, were eligible. Participants were allocated randomly to either red blood cell transfusion or expectant management. Health-related quality of life was assessed at inclusion, three days, and one, three and six weeks post-partum with the Multidimensional Fatigue Inventory (MFI) and the ShortForm-36. The primary outcome was physical fatigue (measured with the MFI questionnaire) three days post-partum. We had hypothesized that there would be no important difference between study arms, using a non-inferiority margin of 1.3. From 2004 until 2011, 494 patients in 37 hospitals were randomized of which 247 were allocated to expectant management and 247 to red blood cell transfusion. Mean hemoglobin at inclusion was comparable between groups (both 7.2 g/dl), as was mean hemoglobin six weeks post-partum (11.8 g/dl, 11.9 g/dl respectively). Seven patients allocated to red blood cell transfusion did not receive transfusion, whereas 31 women allocated to expectant management received red blood cell transfusion, mainly because of anemic complaints. The mean physical fatigue score on day three post-partum was 0.9 higher in women allocated to expectant management (95% CI 0.2-1.6, P= 0.010). After one, three and six weeks, this score was 1.3 (95% CI 0.5-2.0), 0.8 (95% CI -0.1-1.7) and 0.4 (95% CI -0.5-1.3) higher respectively. The differences between study arms were not dependent on mode of delivery or hemoglobin at study entry. We concluded that in women with acute post-partum anemia, red blood cell transfusion led to a small statistically significant decrease in physical fatigue during the first weeks after birth. Therefore red blood cell transfusion should not be routinely prescribed to women with post-partum hemorrhage.

TEMPLE study

The TEMPLE study is set up to improve the treatment of major chronic anemia obstetric hemorrhage. Retrospectively, it compares the effect of currently-used transfusion strategies on clinical patients with chronic anemia.

TEMPOH study

The TEMPOH study is set up to improve (the guidelines for) the treatment of major obstetric hemorrhage. Retrospectively it compares the effect of currently-used transfusion strategies on the clinical outcome of women who received at least 4 units of red blood cells during major obstetric hemorrhage. In addition, a prospective study is being designed to assess hemostasis parameters during ongoing blood loss and clinical outcomes in women suffering major obstetric hemorrhage.

PREPAReS study

The PREPAReS study (Pathogen Reduction Evaluation & Predictive Analytical Rating Score study) is an ongoing non-inferiority randomized trial comparing the clinical efficacy of Mirasol-pathogen reduced platelet concentrates with standard platelet concentrates. The study started in 2011; Erasmus MC has joined the Leiden University Medical Center and HAGA hospital in patients' actual accrual of 69 patients in 2011 and Norway and Canada have made early preparations to contribute to the required intake of 618 patients.
A satellite study to the PREPAReS study was set up to predict bleeding and the effect of platelet transfusions on the risk of bleeding. The first step in this project was to set up a sensitive platelet functionality test for use in both *in vitro* and *ex vivo* samples and a standardized bleeding score that can be graded automatically according to the WHO bleeding grades. The platelet functionality test has now been validated in platelet products after different storage times and compared to several other platelet tests, both well-known and relatively new ones. The test seems to be both sensitive and specific for storage-induced changes in platelet functionality and compares favorably with the other tests (manuscript in preparation). The bleeding score has been used and evaluated on 1187 days in 60 patients (the first interim analysis of the Prepares trial). A computer algorithm was developed and used for the automatic adjudication of WHO bleeding grades. Almost full agreement with three independent human adjudicators was reached. Results will be published after validation in another 60 patients, during the second interim analysis of PREPAReS.
A fundamental platelet study in collaboration with The Brigham and Women's Hospital in Boston was performed.

Key publications

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Heemskerk JW, Sakariassen KS, Zwaginga JJ, Brass LF, Jackson SP, Farndale RW; Biorheology Subcommittee of the SSC of the ISTH. Collagen surfaces to measure thrombus formation under flow: possibilities for standardization. J Thromb Haemost 2011; 9(4):856-8.

Cell therapy

The cell therapy research aims to quantify and weigh the balance between intended and unintended effects of emerging cell therapies.

HOVON study

The ongoing HOVON-106 study investigates whether transplantation of cells of two umbilical cords improves hematopoietic engraftment and the outcome of adults with high risk leukemia or bone-marrow disease.

Neptunis study

The Neptunis study is an observational, prospective, international, multicenter cohort study in patients with long-lasting granulocytopenia who are at risk of invasive infections, such as aspergillosis. The additive role of granulocyte transfusions in these patients is controversial and not proven. The aim of the study is to collect detailed information on patients with hematological disorders requiring treatment causing long-standing granulocytopenia, such as myeloablative stem cell transplantation. The number and type of invasive infections are registered as well as the treatment of these infections with antibiotics and granulocyte transfusions. The primary outcome is the number of patients with invasive infections that would be eligible to receive granulocyte transfusions according to current guidelines. Mortality in this group is also an important endpoint. Other centers involved are HAGA, The Hague; University Hospitals Bristol, NHS Foundation; and Oxford Radcliffe Hospital, NHS Trust.

Cord blood expansion

In this study we investigate the expansion of cord blood cells as part of the stem cell research of Sanquin and LUMC, Leiden. In the future this research will siphon over to Sanquin Research in Amsterdam and will be done in collaboration with the Sanquin Research Department of Experimental Immunohematology.

Key publications

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Blood management and side-effects

The research on blood management and side-effects includes studies set up to compare intended and unintended effects of transfusion and donation strategies, with the aim of guiding the practice of transfusion medicine directly. The studies are either observational or intervention studies, but all have a comparative nature.

TOMAAT study

In 2011 we finished and analyzed the TOMaat study, a randomized study on transfusion alternatives in elective orthopedic surgery. A study examining current practice and barriers for the implementation of the TOMAAT findings under the name LISBOA study (Leiden Implementation Study on BlOodmanagement in Arthroplasty) was started in December 2011 exploring current practice (ZonMW/LUMC/Sanquin project).

FIBER study

The FIBER study assessed the costs and effects of using single-donor fibrin sealant in patients undergoing coronary artery bypass graft surgery, on their need for transfusion and length of stay in the intensive care unit. It is a single-blinded, randomized study among patients undergoing CABG (coronary artery bypass graft) surgery. At the end of 2011 we had included 1,433 patients in 7 centers.

Multiple organ failure

In a case-control cohort of 29 cardiac surgery patients with intermediate risk of transfusion-associated postoperative complications, differences in cytokine gene expression responses after surgery were compared. No RBC transfusion effect on cytokine gene expression could be identified in patients developing post-operative multiple organ failure.

First study

The FIRST study (Fibrin Sealant in Total Knee replacement Surgery Trial) assesses the effect of using single-donor fibrin sealant in patients after a total knee replacement on post-surgical bleeding, swelling and pain. It is a multi-center, semi-blinded study. By flexion and extension as a primary outcome of post-operative knee function, estimated improvements at 2 and 6 weeks after surgery are evaluated. The study requires an intake of 500 patients and at the end of 2011, 147 patients were included from 4 centers. Two other centers will further increase the recruitment in 2012.

TRALI

Ongoing TRALI studies (Transfusion Related Acute Lung Injury) assess the effect of donor, product and patient characteristics on transfusion-related acute lung injury. In 2011 we investigated the effect of storage time of blood products on the risk of TRALI in a case control study. The relative risk of TRALI, after receiving platelets stored for 4 or 5 days, compared to 3 days or less, was 5.8 (95% CI 0.99 to 110) and increased to 6.3 (95% CI 1.1 to 118) after more than 5 days. Storage time of plasma and red cells was not associated with the risk of TRALI.
The presumed deleterious effect of stored red blood cells on the clinical outcome is mainly evaluated in observational studies and a few pilot-randomized clinical trials. Crucial weaknesses in study design and analyses in many of these studies combined with their conflicting results prevent a reliable meta-analysis. As part of the international ABLE (Age of BLood Evaluation) study in high-risk intensive care patients, a Dutch chapter has been set up. In 2011, protocols for both studies were approved by the METC Zuid-Holland, and the screening for inclusion has started in the first hospital, MC Haaglanden. This large international RCT can produce reliable data, where the Dutch RCT also collects data on medium-risk ICU patients.

LUMC red cell storage study

In the Leiden University Medical Center (LUMC) red cell storage study we examined the effect of the storage time of red cells on the mortality of transfusion recipients, in a cohort of successive transfusion recipients in the LUMC. Mortality was compared between recipients of only young blood or only old blood. After correction for various potential confounders, a 1.8-fold (95% CI: 1.0 to 3.1) increased risk of death was observed for recipients of red cells younger than ten days, compared to red cells older than 24 days.

Effects of G-CSF mobilization

In this prospective study we investigate the short-term and long-term effects of the treatment of granulocyte colony-stimulating factor (G-CSF) in related and unrelated stem cell donors. Next to the safety and efficacy of G-CSF, quality of life aspects in these donors are studied. We expect to include the last of 200 subjects in March 2012.
Other ongoing (pilot) study: a comparison of the efficacy of volume-reduced platelet concentrates with that of standard platelet concentrates in neonates.

Key publications

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Sitniakowsky LS, Later AF, van de Watering LM, Bogaerts M, Brand A, Klautz RJ, Smit NP, van Hilten JA. The effect of RBC transfusions on cytokine gene expression after cardiac surgery in patients developing post-operative multiple organ failure. Transfusion Med 2011; 21(4):236-46.

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Donor studies

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The research of the Department of Donor Studies runs along two related research lines:

- Donor Recruitment and Retention
- Donor characteristics and health issues regarding blood donations, including donor deferrals.

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Donor Recruitment and Retention

Behavioral studies

Psychosocial and behavioral aspects of blood donation are the key issues within this research line.
The determinants of donation behavior are important for the donor career. Gaining insight into voluntary withdrawal among new blood donors is important for developing a long-term donor career. Behavioral determinants were measured amongst 5,000 new donors at three different points in their donor career. This longitudinal study provides a thorough understanding of which factors, at what point in time, influence a decrease in motivation leading to early voluntary withdrawal. These insights allow for tailored interventions to improve donor retention. A paper was written which investigated the effects of adverse events (i.e., needle reactions, fatigue and vasovagal reactions (VVR)) and feelings of distress and anxiety on retention of first-time blood donors. Results showed that 9% of donors who experienced an adverse event at their first donation did not return for a second donation.
A second paper was written on the effect of VVR or needle reactions in regular donors, and the effect on stopping risk, taking into account several behavioral determinants from the Theory of Planned Behavior (TPB). Is stopping risk related solely to the adverse reaction itself, or do the TPB variables also play a role? Emphasis was placed on possible sex differences. The results of this study showed that female donors report more VVR than male donors, but that male donors have a twofold higher stopping risk after a VVR than female donors. Coping and reporting tendency differences might play a role. For donor retention purposes, prevention and coping techniques should take sex differences into account.
In a third paper, it was investigated whether lapsed donors would be willing to re-start donating. Research among 800 lapsed donors showed that the majority of lapsed donors indicated a moderate to high intention to restart donations. Interventions focusing on boosting cognitive and affective attitudes and self-efficacy could further raise such intentions.
In 2009 a PhD project started on show/no-show behavior of donors after receiving an invitation to donate. In 2010 information was gathered about no-show behavior of donors in different ways, namely: exploring existing literature for a review article, analyses of cohort data among blood donors (Donor InSight research), and analyses of blood bank data to calculate show-rates in different ways. In cooperation with the donor administration, a study was also designed to clarify how many donors cancel their invitation for a donation and with which reason. Additionally, 90 donors were interviewed about their satisfaction with the invitation system, reasons for previous no-show behavior and negative donation experiences. Questionnaire data have been gathered among 2,500 donors on different topics related to show behavior. Two papers are currently being written on the initial results.

DOnor MAnagement IN Europe, DOMAINE

DOMAINE (Donor Management IN Europe) is a European Union co-funded project, in which blood establishments from 18 European member states and one patient-driven organization join forces on donor management.
DOMAINE aims to compare and recommend good donor management practice. It focuses on various aspects of donor management (including cultural differences): donor recruitment strategies, donor retention strategies, deferral procedures and blood bank policy regarding patients requiring long-term transfusion. In 2008/2009, a survey was conducted to analyze donor management practice in Europe. In total, 48 questionnaires were sent to 37 European countries, with a response rate of 88%.
The (confidential) survey report was finalized in 2009. The report has served as a template for the manual on good donor management, published in June 2010.
Finally, in 2011 the DOMAINE group presented a training program for blood donor management professionals, based on the manual.

Key publications:

De Kort W, Veldhuizen I (Eds.). Donor Management Manual 2010. DOMAINE project. DOMAINE, Nijmegen, the Netherlands, 2010. ISBN 978-90-815585-1-8.

De Kort W, Wagenmans E. Donor management – ethics, culture and minority involvement in the blood supply. ISBT Science Series 2011; 6:223–8.

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Donor characteristics and donor deferrals

Donor characteristics and health effects related to blood donation are the main issues within this research line. There is also a strong focus on donor deferral.

Donor Characteristics and health effects of blood donation

The research in this field focuses on health and disease within donors. The donor population is said to reflect a relatively healthy subset of individuals. In order to gain insight into the health status of donors, a description of the donor pool in terms of demographic characteristics and cardiovascular risk factors has been made, using Donor InSight data and data from the general population as a reference. The methodological consequences of this healthy donor effect in donor health research have also been investigated. When comparing blood donors with the general population or active donors with lapsed donors, substantial bias may be introduced. In the case of internal comparisons (high-frequent donors with low-frequent donors within a group of active donors) this healthy donor bias appeared to be less. We therefore recommended embedding donor health research within an active group of donors.
Additionally, a PhD project on donation frequency and cardiovascular disease was started at the end of 2009. This study is carried out within donors. The occurrence of cardiovascular disease is explored by linking donation data from current and ex-donors with morbidity and mortality data from Statistics Netherlands. The effect of the number of donations on metabolic factors, such as blood pressure, lipid levels, and insulin sensitivity, is also investigated within active and newly registered donors. To this end, information about cardiovascular risk factors from 630 active donors and 120 newly registered donors was collected in 2011. The newly-registered donors will return for a second measurement after two years of follow-up.

Donor deferral

Low hemoglobin (Hb) level is an important reason for donor deferral. In the Netherlands, in total, about 10% of the donors visiting a collection center, are deferred. Within the deferred group, 2-3 % of male donors up to 5-7 % of female donors are being deferred for low Hb. Since deferral is a proven reason for donor lapse, reducing this percentage is paramount. Hb is known to be related to several factors, including gender, physical condition, iron status, Body Mass Index, nutrition, but also environmental conditions, such as environmental temperature and donation history. To disentangle these complex relations we recently started an extensive statistical modeling study on prognostic factors of Hb. The first modeling results in a small cohort have been published (Baart et al., 2011). In 2011 a model study on a large cohort was assessed (Baart et al., accepted for publication). In a pilot study among donors, substantial iron depletion – measured as Zinc Protoporphyrin (ZPP) levels – was observed. ZPP is an anticipated predictor of iron-depleted Hb production.

Key publications

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Atsma F, Veldhuizen I, Verbeek A, de Kort W, de Vegt F. Healthy donor effect: its magnitude in health research among blood donors. Transfusion 2011; 51(8):1820-8.

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Peffer K, de Kort WL, Slot E, Doggen CJ. Turbid plasma donations in whole blood donors: fat chance? Transfusion 2011; 51(6):1179-87.

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Product and Services Departments

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Product Development Plasma Products

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The product development strategy of Sanquin Plasma Products aims primarily at maintaining the state-of-the-art level of its plasma derivatives portfolio and production processes. To that end, the product and process development program is regularly evaluated and updated if needed. Besides, opportunities for development of new (plasma) products are being explored in feasibility studies which may evolve into full-blown development projects when considered to be economically feasible.

Our activities:
- Development of new products
- Improvement of plasma products
- Characterization new indications

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The goal of the CAF-DCF Product Development Department (R&D) is to ensure both the pre-clinical and clinical efficacy of plasma-derived medicinal products and their biological safety regarding pathogens, pollutants, and accompanying proteins. Focusing on therapeutic proteins (IVIgs, albumin, fibrinogen, AGP, FVIII) in starting plasma pools and final concentrates, the Department has developed both immunological methods and biochemical-biophysical techniques, including predictive epitope-mapping algorithms for FVIII proteins and B19 NS1 and capsid proteins. Results are exploited in industrial applications (20 patents granted in the EU and US, of which 3 patents in 2011).

Recently, several lots of intravenous immunoglobulin (IVIg) produced by other manufacturers have been implicated in thromboembolic events (TEEs) in patients. An investigation was launched to detect possible procoagulant/coagulant contaminants in in-process fractions and IVIgs produced by the CAF-DCF, Sanquin, and other manufacturers. Emphasized was developing and validating a battery of methods, particularly for FXIa, believed to be the major cause of the TEEs. In in-process fractions, FXII/FXIIa, FXI/FXIa, thrombin, prekallikrein/kallikrein, and plasminogen/plasmin were found, but no pro-/coagulant activity was detected in the final IVIg products of CAF-DCF and Sanquin, Multigam® or Nanogam®, respectively, even after concentrating the accompanying proteins.

Invasive pneumococcal diseases in children persist as a major cause of morbidity and mortality worldwide. In a multi-center clinical study, the protective role of IVIg administration was evaluated in 22 pediatric primary immunodeficiency patients by quantifying antibodies specific for 16 pathogenic serotypes of *S. pneumoniae* in patient sera and in the administered product (Multigam®).

The results demonstrated statistically significant correlations of plasma levels after IVIg infusion in patients with anti-serotype antibody levels in the administered plasma product and with the complexity of the epidemiology of *S. pneumoniae* among the donors from whom plasma was collected.

The paradigm of plasma derivative safety is approached through different routes: NAT screening, statistical evaluation of critical virus epidemiological data, neutralization by specific antibodies, virus infectivity testing in a cell model, virus inactivation/elimination validation studies, and pathogen reduction techniques (including UVC irradiation developed in our Department).

Our activities include:
- Infection protective antibodies and sero-epidemiology
- Pneumococcal Ig in IVIg-treated infants, in relation to the standard for vaccines
- (Pro-)coagulant activities in intermediate Cohn fractions and IVIgs
- Albumin formulated with caprylate with or without N-Ac-tryptophan in a liver support device (Mars)
- B19 proliferation in hepatoblastoma cells
- Epidemiology of viral markers in donors in Belgium
- A new FVIII/VWF concentrate with 3 virus inactivation steps

Medical Department Sanquin Plasma Products

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The Medical Department is, in its applied research activities, responsible for the design and conduct of clinical trials with (recently developed) plasma products. The Medical Department closely cooperates with clinical investigators in the Netherlands e.g. the Netherlands Inter-University Working Party on the Study of Immune Deficiencies and the Dutch Haemophilia Treatment Centers, and with clinical investigators and researchers abroad. In 2011, three clinical studies with intravenous immunoglobulin, Nanogam®, were ongoing in order to study the efficacy and safety of Nanogam® in different clinical conditions. Clinical studies with apotransferrin, Cetor (C1-esterase inhibitor concentrate) and Cofact (Prothrombin Complex Concentrate) were also initiated, and preparations were made for a number of clinical studies with a newly developed FVIII product.

Reagents

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Sanquin Reagents has developed a broad range of blood grouping and immunology reagents for laboratories, including several innovative products for diagnostic use and for clinical research. These reagents are available worldwide through a network of distributors, and bulk reagents for manufacturing are supplied directly from Amsterdam. Reagents is ISO 9001 and ISO 13485 certified and is committed to introducing new products on a continuous basis. New products are the outcome of R&D projects, some of which are executed in close collaboration with research departments within Sanquin and/or with other companies and institutions.

Pharmaceutical Services

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Sanquin Pharmaceutical Services (SPS) is a business unit specialized in a broad array of pharmaceutical services aiming at the development and quality testing of biologicals intended for therapeutical application in humans. These services include the development of adequate production processes, contract production of mammalian cell products (monoclonal antibodies and/or r-DNA) as well as safety testing and designing validation studies for assays and processes, including viral reduction studies.
Licenses and accreditations:

- GMP license for production activities
- GCLP license as part of GMP for all QC and safety tests
- GLP accreditation for viral and prion validation services

Diagnostic Services

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Sanquin Diagnostic Services excels in routine and top-reference specialized testing in the field of blood-related diseases and immune-mediated disorders. The blood sample testing is carried out in Amsterdam and is available to all healthcare institutions and commercial companies in the Netherlands and abroad. The division aims to work in accordance with the highest quality standards so as to function as a diagnostic reference center in the fields mentioned above, in both national and international settings. With its fully certified laboratories, Sanquin Diagnostic Services can provide a vast array of both routine and tailor-made diagnostic tests. Sanquin Diagnostic Services is committed to continuous innovation reflected by the introduction of new diagnostic tests. New tests are often developed and validated in-house, in R&D projects, most of which are carried out in close cooperation with Sanquin Research.

Consulting Services

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The mission of Consulting Services (SCS) is to provide guidance and advice services to restricted economy countries.
Objectives are:
- To support restricted economy countries in developing safe, efficacious and sustainable blood supply systems based on current quality principles,
- To provide modular training programs on transfusion medicine for restricted economy countries focused on the managerial and quality aspects of the transfusion chain, and
- To extend and strengthen the training and consultative potential within the Sanquin organization.

Education

Through educational programs supported by the Academic Institute for International Development of Transfusion Medicine (IDTM) we are able to award a Master of Management in Transfusion Management. This institute is part of the University of Groningen (RUG)/Faculty of Medical Sciences and the University Medical Center Groningen (UMCG), under the umbrella of the WHO Department of Essential Health Technologies.
In collaboration with IDTM, educational (e-learning) programs in the field of transfusion medicine were developed. IDTM and Consulting Services both contribute to the education program Master in Management of Transfusion Medicine. The Academic Institute IDTM is responsible for and provides an academic education, consisting of a master thesis and e-learning program of 12 months. Consulting Services supports a fellowship in the Sanquin Blood Bank in The Netherlands for a period of 6 months.

Publication

Los APM, Mugisha W, Ayikanying M, Mutegombwa SM, Kyeyune-Byabazaire D, Smid WM. Meeting stability and continuity of the blood supply over the year: a unique approach combining the liaison role of school teachers with the establishment of community resource persons- could the Uganda experience serve as an example for other African countries? Africa Sanguine 2011; 14 (2):3-6.

Patent portfolio and valorization

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In addition to our efforts to publish our research work in scientific publications, Sanquin also disseminates knowledge in the form of patents and other types of know-how. In 2011 Sanquin focused its IP efforts on the enhancement of the clinical efficacy of biologicals in the area of blood coagulation factors, intravenous immunoglobulin (IVIg) and C1-esterase inhibitor; monoclonal antibodies and cellular vaccines.
Most often in-licensing parties seek both the opportunity of patent protection for future product pipelines, and the expertise of our inventors and their research groups. So a license contract generally incorporates a joint research agreement enabling Sanquin to generate funding for its research and enabling third parties to evaluate the patent proposition.
An overview of the valorization status of Sanquin Research patents and hybridomas is shown. Most patents/hybridomas listed have a primary therapeutic application.

Patents/Patent Applications	**Status 2011**
Enhancement efficacy blood coagulation factors amongst others	
'FVIII mutants'	3rd party out-licensed
'FVII-LRP antagonists'	Licensing discussions ongoing
'Anti-FVIII antibodies' (improving half-life)	Open for licensing
'C1-esterase inhibitor in AMI'	3rd party out-licensed
'IVIg (improving therapeutic efficacy)	Open for licensing
Enhancement efficacy monoclonal biologicals	
'Antibody Stability' (improving therapeutic efficacy IgG3)	Open for licensing
'SIRPalfa interference' (improving ADCC efficacy)	Open for licensing
'Anti-antibody' (detecting antibodies against therapeutic Abs)	Discussions ongoing
'Monitoring of Immunoglobulin receptor genes'	Open for licensing
Enhancement Cellular Therapies & Vaccine Development	
'Hobit-transcription factor for killer cell activation'	Open for licensing
'MHC Multimers'	Open for licensing

Sanquin hybridomas	**Status 2011**
RAG_35_201	Open for licensing
CD 97	Open for licensing
CD3 human IgM, IgG1, IgG2, IgG3, IgG4, IgA, IgE	Open for licensing
Anti c-1q / Anti c3-2 / 2C8	Open for licensing
4-7B	3rd party licensed semi-exclusively
CD70	3rd party licensed
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Miscellaneous Publications

Ágoston SMI, van Mourik PC, Strengers PFW. Quality risk management: a valuable tool in implementing, maintaining and improving a quality management system. ISBT Science Series 2011; 6:52-5.

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Theses 2011

Simon van Haren
22 December 2011
Immune recognition and processing of blood coagulation factor VIII by antigen-presenting cells
Promotor: Prof K Mertens PhD
Co-promotores: J Voorberg PhD and AB Meijer PhD
University of Utrecht

Jos van Rijssel
21 December 2011
Docking onto the endothelium - Trio directs leukocyte extravasation
Promotor: Prof D Roos PhD
Co-promotores: JD van Buul PhD and Prof PL Hordijk PhD
University of Amsterdam

Marijke Maijenburg
12 October 2011
Characterization of human mesenchymal stromal cell heterogeneity
Promotor: Prof CE van der Schoot MD PhD
Co-promotor: C Voermans PhD
University of Amsterdam

Yavuz Bilgin
28 September 2011
Transfusion-associated complications in cardiac surgery: the 'swan song' of the allogeneic leukocytes?
Promotor: Prof A Brand MD PhD
University of Leiden

Eveline Bouwens
31 August 2011
Factor VIII and von Willebrand factor co-delivery by endothelial cells
Promotor: Prof K Mertens PhD
Co-promotor: JJ Voorberg PhD
University of Utrecht

Henriët Meems
31 August 2011
New insight into the C1 domain of coagulation factor VIII
Promotor: Prof K Mertens PhD
Co-promotor: AB Meijer PhD
University of Utrecht

Sebastiaan Bol
7 July 2011
Host genetic effects on HIV-1 replication in macrophages
Promotor: Prof H Schuitemaker PhD
Co-promotor: AB van 't Wout PhD
University of Amsterdam

Daniëlle van Manen
24 June 2011
The influence of host genetic factors on HIV-1 infection
Promotor: Prof H Schuitemaker PhD
Co-promotor: AB van 't Wout PhD
University of Amsterdam

Janine Stutterheim
16 June 2011
Minimal residual disease detection and monitoring in children with neuroblastoma
Promotores: Prof HN Caron MD PhD and prof CE van der Schoot MD PhD
Co-promotor: GAM Tytgat PhD
University of Amsterdam

Ineke Rood
7 April 2011
Detection and origin of bacteria in platelet concentrates
Promotor: Prof PHM Savelkoul PhD
Co-promotores: D de Korte PhD and AM Pettersson PhD
Free University of Amsterdam

Marit van Gils
1 April 2011
Cross-reactive neutralizing humoral immunity in HIV-1 disease: Dynamics of host-pathogen interactions'
Promotor: Prof H Schuitemaker PhD
University of Amsterdam

Diana Edo Matas
21 January 2011
Within-host HIV-1 evolution in relation to viral coreceptor use and host environment
Promotor: Prof H Schuitemaker PhD
Co-promotor: AB van 't Wout PhD
University of Amsterdam

Rutger Middelburg
19 January 2011
Transfusion-related acute lung injury. Etiological research and its methodological challenges'
Promotor: Prof E Briët MD PhD
Co-promotor: JG van der Bom MD PhD
University of Leiden

Micha Nethe
14 January 2011
Rac1 meets ubiquitin. New insights in Rac1 signaling
Promotor: Prof D Roos PhD
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