

Scientific Report 2006

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Words of welcome

It is with pleasure that I may once again present to you our annual scientific report.

In this 2006 scientific report you will find some information on our organization and our policies towards research. Of course, all our research lines are described, with some key publications for further reference. All research departments are listed with staff, students and contact information. For the first time, we have included an overview of our patent portfolio and licensing opportunities.

Since the merger in 1998 of CLB with the blood banks into the Sanquin Blood Supply Foundation our research program is moving from basic immunology towards more blood supply related research. Nevertheless, we still strive to keep at least thirty percent basic research in our research and development program.

In 2006 the research programs at our Blood Bank research departments was further strengthened. The department of Research and Education of the South East Region is concentrating more and more on donor related studies, with expertise from epidemiology and the social sciences, in order to have our donor policies scientifically based. The South West Region is concentrating on clinical studies in close collaboration with academic hospitals, especially Leiden University Medical Center, as well as the larger general hospitals in the Netherlands. The North West Region is dedicated towards transfusion technology in close collaboration with Sanquin Research. In the North East Region we are discussing new lines of research with the Groningen Academic Medical Center.

Sanquin Research is continuing its research on immunology, hematology and infectious diseases within the joint Sanquin / Academic Medical Center, University of Amsterdam Landsteiner Laboratory.

Our collaboration with Utrecht University on coagulation and transfusion technology assessment was continued.

In 2006 professor Dirk Roos retired, at which occasion a farewell symposium (The Roos' line of defense) was organized that was very well attended. Professor Roos' research group on granulocyte function will continue under the new leadership of Timo van der Berg, who joined Sanquin Research early in 2007. The collaboration with Emma Children's Hospital / Academic Medical Center, University of Amsterdam will of course be continued as well.

At this years annual conference of the International Society of Blood Transfusion (ISBT) dr. Masja de Haas of Sanquin Research & Sanquin Diagnostic Services was awarded the prestigious Jean Julliard Prize.

In November 2006 we organized the Sanquin Science Day ('Wetenschapsdag'). At this symposium all PhD students from Sanquin presented their research with over 65 posters and senior scientists of Sanquin gave presentations on their most valued research topic. At this Science Day, three nominees for the Sanquin PhD award, presented their research. The award was won by Jean Paul ten Klooster for his innovative research on RAC-1 induced cell migration and his excellent presentation. The other nominees were Sandra Cauwenberghs and Magdalena Lorenowicz. We intent to organize this Science Day every two years alternating with the biennial Sanquin Spring Seminars.

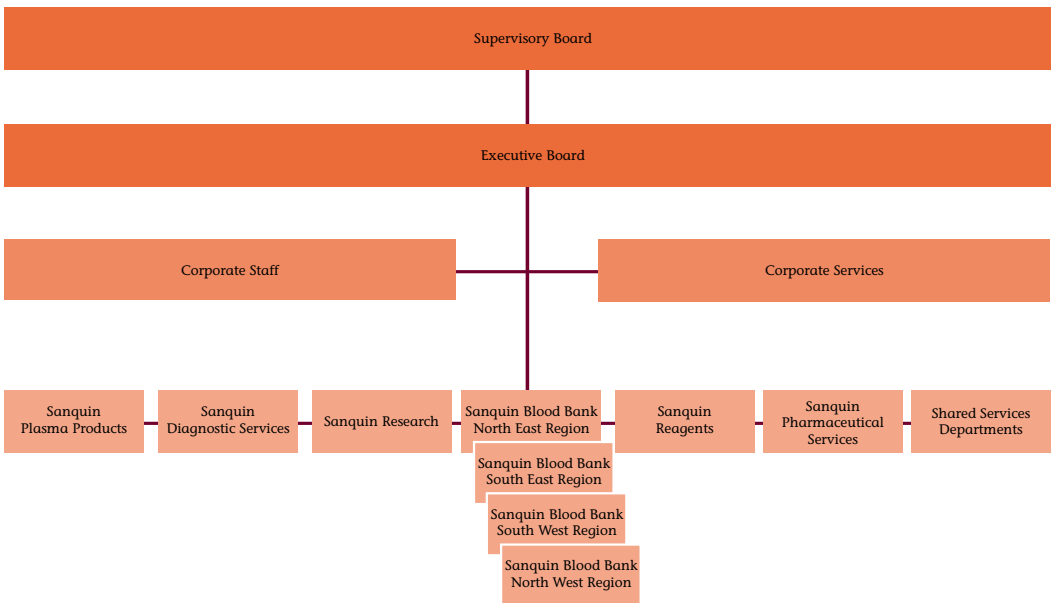
I hope you will find this 2006 scientific report worth while to read and to have as a reference guide to Sanquin's research program and our dedicated research staff.

Prof Ernest Briët MD PhD
Member Executive Board

Introduction

Sanquin Blood Supply Foundation

Sanquin Blood Supply Foundation consists of seven divisions and two business units. 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. Four Blood Bank divisions each, have their own department of Research and Education. Finally, at the Amsterdam premises we find Sanquin Diagnostic Services, Sanquin Plasma Products, Sanquin Research, and the business units Sanquin Pharmaceutical Services and Sanquin Reagents.



Research Programming Committee

The Research Programming Committee is advising the Executive Board on strategic issues and on selection of projects funded from Sanquin's own resources. A yearly call for proposals on product and process development is issued, and projects are reviewed by international referees before selection to guarantee the quality of the research proposals.

In 2006 actions were continued to improve the quality of research proposals to be submitted to external funding agencies and charities by internal review meetings and procedures.

The Research Programming Committee consisted of five members representing four product/market combinations: Blood Banks (Prof DJ van Rhenen MD PhD, HJC de Wit PharmD), Plasma Products (PFW Strengers MD), Diagnostic Services (C Aaij PhD) and Research (Prof E Briët MD PhD), supported by an executive secretary, JW Smeenk MSc).

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. Furthermore, the Scientific Advisory Board assesses the quality of Sanquin's research based on bibliometric analyses and reports of site visits.

On 31 December 2006 the Scientific Advisory Board consisted of:

Prof E Briët MD PhD (Chairman, Sanquin Executive Board & Universities of Amsterdam and Leiden)

Prof A Brand MD PhD (Sanquin Blood Bank South West Region & Leiden University)

C de Visser PhD (Netherlands Organisation for Scientific Research)

Prof RRP de Vries MD PhD (Leiden University)

Prof DE Grobbée MD PhD (Utrecht University)

Prof MM Levi MD PhD (University of Amsterdam)

Prof DKF Meijer PhD (University of Groningen)

Prof DJ van Rhenen MD PhD (Sanquin Blood Bank South West Region & Erasmus University Rotterdam)

JW Smeenk MSc, Executive secretary (Sanquin Corporate Staff)

Research Assessment

In de December 2006 Sanquin's research in the area of transfusion transmittable infections was assessed by an international peer review committee. This site visit resulted in a number of recommendations on strengthening research management

as well as an assessment of the quality and relevance of research projects. The peer review committee consisted of prof Jussi Huttunen (Finland) who acted as chairman, prof Dana Devine (Canada), dr Thomas Montag Lessing (Germany), and prof Rudi Westendorp (The Netherlands). The Committee was supported by dr Roel Bennink of QANU (Quality Assurance Netherlands Universities), which made it possible to better tune our quality assessment system to that of Dutch academia.

All research groups of Sanquin are visited by a peer review committee once in every five years. In earlier years these site visits were organized by departments; following the advice of the Scientific Advisory Board, this years' site visit was organized thematically, basically in line with the research lines presented in this report.

Academic affiliations, education and training

Sanquin research departments attract many students who participate in scientific projects. Historically there is a strong collaboration with the Academic Medical Center (AMC) of the University of Amsterdam. This joint AMC – Sanquin Landsteiner Laboratory is housed within Sanquin premises.

At many Dutch universities, staff from various Sanquin divisions is 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 of specialists in blood transfusion medicine, other medical specialties, and training of nurses.

Sanquin has established a recognized training program for medical doctors specialising in transfusion medicine and/or blood 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 North East Region runs a postgraduate masters program, under the heading of Academic Institute for International Development of Transfusion Medicine (IDTM). Sanquin is WHO Collaborating Organisation for Transfusion Medicine.

Professorships Sanquin Staff

Prof Rob Aalberse PhD (Biological immunology, Subfaculty of Biology, University of Amsterdam)

Prof Lucien Aarden PhD (Molecular immunology, Academic Medical Center, University of Amsterdam)

Prof Anneke Brand MD PhD (Blood transfusion medicine, Leiden University Medical Center)

Prof Ernest Briët MD PhD (Epidemiology of blood transfusion, Leiden University Medical Center and Medicine, Academic Medical Center, 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 Dick van Rhenen MD PhD (Blood transfusion medicine, Erasmus University Medical Center, University of Rotterdam)

Prof Dirk Roos PhD (Non-specific immunology, Academic Medical Center, University of Amsterdam)

Prof Hanneke Schuitemaker PhD (Virology, especially viro-pathogenesis of AIDS, 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)

Landsteiner Laboratory

As mentioned above, there is a long standing collaboration with the University of Amsterdam in the joint AMC-Sanquin Landsteiner Laboratory. Through this collaboration Sanquin staff members participate in research programs and curricula of the AMC Research Institute for Immunology (JJ van Loghem Institute) and the Research Institute for Infectious Diseases. Sanquin staff members are heading the following research themes of the AMC research program:

- Infections, Sepsis and Inflammation Processes (prof D Roos PhD)
- Autoimmune Diseases, Allergies, Immunodeficiencies (prof RC Aalberse PhD)
- Immunogenetics & Transplantation Immunology (prof CE van der Schoot MD PhD)
- Virus and host factors in AIDS pathogenesis (prof J Schuitemaker PhD)

During 2006, the AMC reorganized its research organization from research programs to a system of principal investigators. Sanquin and AMC continue the discussions on further strengthening and extending collaboration within the Landsteiner Laboratory, keeping in mind the changes in research management at AMC.

Accreditation and quality assurance

Code of conduct

In 2006 Sanquin Executive Board decided on a research code of conduct, that is based on various codes of conduct from Dutch Universities and de Royal Netherlands Academy of Arts and Sciences. Sanquin applied to join LOWI, the national organization for scientific integrity, that will act as independent advisory body in case of a breach of scientific integrity by a Sanquin member of staff. An independent ombudsman was already appointed.

Accreditation

During 2006 all laboratories of Sanquin Research have been subject to an internal audit by the QOE (Quality, Occupational Health and Environmental safety) department. The scope of these audits was ISO 17025 as explained in T31 by the Dutch Accreditation Council, ISO 14001 and OHSAS 18001.

The departments of Virus Safety Services, Clinical Monitoring and Blood Transfusion Technology were visited by the Dutch Accreditation Council (RvA) and the CCKL in April 2006, and prolonged their accreditation according to ISO 17025 and certification according to the CCKL Code of practice version 4 with another four years.

The laboratory for Stem Cell Transplantation held its certification to ISO 9001 and ISO 13485 as it was successfully visited by the Lloyds auditor. An audit team of JACIE (Joint Accreditation Committee ISCT & EBMT) and CCKL also inspected the laboratory for Stem Cell Transplantation and granted a certificate to the Standards for Haematopoietic Progenitor Cell Collection, Processing & Transplantation and the CCKL Code of practice version four.

Virus Safety Services held its Endorsement of compliance with the OECD GLP principles.

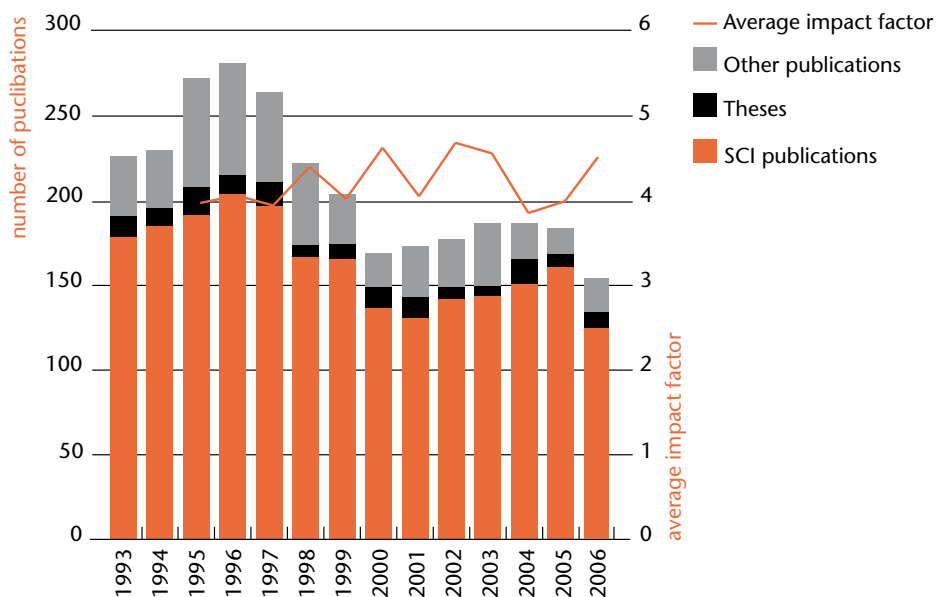
Publications

Due to the fact that the research groups of Miedema and Van Ree have moved to a university setting, the number of papers in peer reviewed journals was less than in previous years. The average impact factor, however, was higher. The number of citations in the five years after publishing (2001) was 2200 for 131 papers, an average of 16.9 citations per paper.

Scientific publications

Year	Total number	SCI publications	Theses	Average impact factor
1993	226	179	12	na
1994	230	185	11	na
1995	272	192	16	4,0
1996	281	204	11	4,1
1997	264	197	14	4,0
1998	222	167	7	4,4
1999	204	166	9	4,0
2000	169	137	12	4,7
2001	173	131	12	4,1
2002	177	142	7	4,7
2003	187	144	6	4,6
2004	187	151	15	3,9
2005	185	161	8	4,0
2006	157	126	8	4,5

Scientific publications and average impact factor



Articles* published in 1993 through 2001 annual reports cited** in five full years after publication

<i>Publications from year</i>	<i>Total citations</i>	<i>Number of SCI publications</i>	<i>Average number of citations per publication</i>
1993	3483	179	19,5
1994	3599	185	19,5
1995	3215	192	16,7
1996	3057	204	15,0
1997	2962	197	15,0
1998	3448	167	20,6
1999	2910	166	17,5
2000	2699	137	19,7
2001	2220	131	16,9

* Only SCI publications are included

** Excluding self citations

Articles* published in 1993 through 2001 annual reports cited** in five full years after publication

Citations in year

	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
1993	551	800	818	699	615								
1994		596	894	871	686	552							
1995			484	736	732	641	622						
1996				491	736	685	615	530					
1997					369	661	657	656	619				
1998						646	811	768	646	577			
1999							468	726	677	543	496		
2000								442	614	580	552	511	
2001									349	510	513	434	414

* Only SCI publications are included

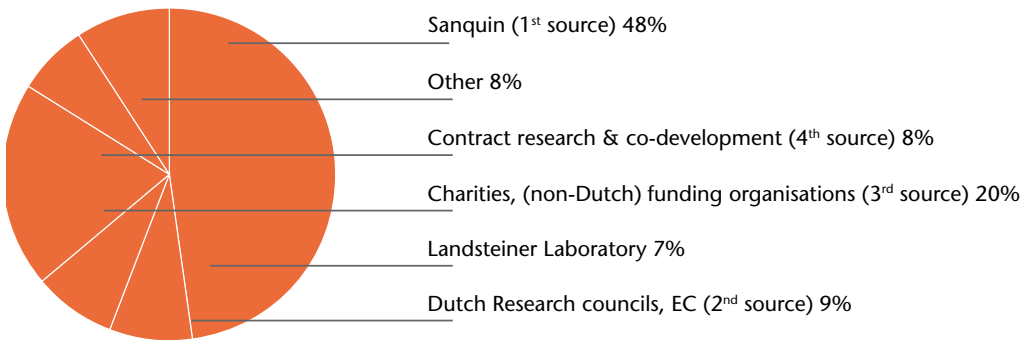
** Excluding self citations

Funding

In 2006 Sanquin researchers were again successful in obtaining external funding (See page 153 for an overview of our sponsors). Various research groups were successful in funding from the sixth Framework Program of the European Community. As European Research funds become more and more important, Sanquin invests in external consulting services to assist our staff in forming consortia and writing proposals for the seventh Framework Program that will start in 2007, and to assist in administrative and organizational matters. Contract Research income was comparable to that in the years before.

Twelve research projects were funded from Sanquin resources for product and process development for cellular products, after a review on quality by external experts and relevance to Sanquin's mission by the Research Programming Committee. Unfortunately over fifteen good proposals could not be funded, due to lack of resources.

Sources of funding of research projects (direct costs only)



Valorization

In 2006 Sanquin started with a new policy to stimulate researchers to file patents. Income from patent or licensing forms additional funding for research. In addition, Sanquin licenses or sells cell lines and monoclonals. On page 151 you will find an overview over the last five years of patents filed and cell lines and monoclonals available, with their current status.

Research lines

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Hematology

Alloimmunization against blood group antigens

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In a collaborative European study (BloodGen) the first prototype of a so-called 'blood-chip' was launched during the ISBT-meeting in Capetown, September 2006. Also during this meeting Dr Masja de Haas has been awarded with the Jean Julliard prize, for the work that has been the basis of this European project. The humoral immune response against RhD has been studied at single cell level. Intriguingly we found that in the peripheral blood of hyperimmunized anti-D donors a large memory B-cell pool against the RhD antigen exists in an IgM+ CD27- negative B-cell subset. However, this subset was found to be negative for the ABCB1 transporter, that discriminates naïve from memory cells. The previously observed overrepresentation of HLA-DR15 in hyperimmunized anti-D donors was not found in pregnant women with anti-D, suggesting that HLA-DR15 is more associated with the magnitude of response than with respondership. To study the working mechanism of anti-D immunoprophylaxis we will make RhD transgenic mice. In 2006 we finished the construction of vectors needed for the generation of these transgenic mice. The study on the efficiency of antenatal anti-D immunoprophylaxis has been finished. As reported last year antenatal prophylaxis reduced the incidence of RhD alloimmunization as measured in the first trimester in the following pregnancy with 50%. However, we observed that in women with failure of ante- and postnatal prophylaxis the RhD antibodies were more often detected later in pregnancy than in the group with only postnatal prophylaxis. Moreover, the risk to develop severe HDFN in RhD-immunized pregnancies, detected upon 30th week screening, was significantly lower in the intervention group compared to the control group. These data suggest that the addition of antenatal prophylaxis has a long lasting suppressive effect of the magnitude of the immune response. In the European Network of Excellence (SAFE) the application of new technological approaches for non-invasive prenatal genotyping on fetal DNA present in maternal blood is further investigated. The IgG transport by FcRn (the main receptor responsible for the transport of maternal IgG across the placenta) has been studied *in vitro*. Despite the differences in half life of IgG1 and IgG3 in plasma due to difference binding characteristics of these IgG's of FcRn, a similar transport rate was observed

in vitro. However, in the presence of IgG1 and IgG3 the IgG3 transport rate is inhibited. By making mutants IgG1 and IgG3 we could demonstrate that this effect was dependent on the presence of His and Arg at position 435 in IgG1 and IgG3, respectively. To get insight in possible mechanisms underlying the pathogenetic effect of anti-HPA-1a antibodies on fetuses, we tested the effect of these antibodies on endothelium.

Key publications

Dohmen SE, Verhagen OJ, de Groot SM, Stott LM, Aalberse RC, Urbaniak SJ, van der Schoot CE. The analysis and quantification of a clonal B cell response in a hyperimmunized anti-D donor. *Clin Exp Immunol* 2006; 144(2):223-32.

Vidarsson G, Stemerding AM, Stapleton NM, Spliethoff SE, Janssen H, Rebers FE, de Haas M, van de Winkel JG. FcRn: an IgG receptor on phagocytes with a novel role in phagocytosis. *Blood* 2006; 108(10):3573-9.

Molecular blood group polymorphisms

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The aim is to evaluate the application of molecular blood group typing of red cell and platelet antigens for blood bank purposes (donor typing) and for diagnostic and therapeutic purposes.

Medium and high throughput RBC genotyping

Multiplex PCR and multiplex Pyrosequencing RBC assays have been developed to type for KEL1/KEL2, KEL3/KEL4, MNS1/MNS2, JK12 and FYX. Single assays are FY1/FY2, FY-GATA-1 and LU1/LU2. Genotyping results were evaluated among four different ethnic groups, (i.e. blacks from South Africa (BSA), Asians from South Africa (ASA), blacks from Ethiopia (BE) and Caucasians from The Netherlands (CN)), by comparing genotyping results with serological results. Pyrosequencing results were in concordance with serological results for Kk (123 ASA, 127 BSA, 99 BE, 49 CN) KpaKpb (125 ASA, 126 BSA, 107 BE, 49 CN), Jk (113 ASA, 126 BSA, 94 BE, 47 CN), Fy (94 ASA, 88 BSA, 50 BE, 46 CN), MN (111 ASA, 106 BSA, 83 BE, 37 CN) and Lu (118 ASA, 114 BSA, 94 BE, 49 CN). One FYX-allele was detected next to a normal FY2 allele in 1/50 CN and in 1/98 BE a FYX-allele was detected next to a silenced FY2-allele (GATA-1 mutation). All genotypes determined by Pyrosequencing proofed to be correct. Discrepant samples were confirmed by PCR-SSP, DNA-sequencing or both. 5 discrepancies between serology (antigen-negative) and genotyping (presence of SNP encoding the 'missing' antigen) were found. Those discrepancies need further investigation. Genotyping by Pyrosequencing on the SNPs for KEL1/KEL2, KEL3/KEL4, JK1/JK2, FY1/FY2 in combination with GATA-1, FYX, MNS1/MNS2 and LU1/LU2 to predict Kk, KpaKpb, Kidd, FyaFyb, MN and Lu phenotypes seems reliable in a multi-ethnic society and in contrast with what may have been expected, no discrepancies due to for instance null-alleles, were found.

Within an European fifth framework demonstration project, a so-called Bloodchip has been developed which was launched at the ISBT 2006 in Cape Town. A large cohort of individuals drawn from across the EU was genotyped in order to demonstrate the accuracy and improvement of molecular genetic techniques over standard serological testing.

Genotyping of D-negative donors

To establish the frequency of RHD positive alleles in serologically typed D-negative donors two molecular techniques, Pyrosequencing- and TaqMantechology, are being used to screen donor pools of D-negative donors. For both techniques it has been shown that the presence of one donor sample with a chimaeric RHD status of only 1% RHD-positivity can be detected in a pool of 24 RhD-negative donors. The number of D negative and C and/or E positive samples that has been collected and analyzed till December 2006 is 831, i.e. 510 C positive, 315 E positive and 6 CE positive. In addition 4931 D negative and CE negative (rr) samples have been collected and analyzed. 754 samples still need to be investigated. Of every sample in which an RHD allele was found, results were confirmed with a second sample, which was also used to extent the molecular typing by RHD-exon-specific sequencing. Summary of results:

D neg	Total number	RHD-gene present	RHD-allele causing weak D expression	Cause of weak D expression
C pos	510	12	4	1x Del; 1x weak D type 30 1x weak D type 48; 1x chimera
E pos	315	5	1	1x weak D type 30 (needs to be confirmed)
CE pos	6	2	2	1x weak D type 2 1x chimera
ccee	4931	2	1	1x chimera

Because fewer donors with (very) weak D expression (negative upon routine serological analysis) have been identified than was estimated based on results in literature, it will be calculated whether an informative prospective study on the risk of immunization upon transfusion of these units will be feasible. Ethical considerations will be taken into account as well. In addition it will be investigated whether an informative look-back study can be performed, tracing D negative patients who received blood from the 8 donors found in this study so far.

Key publications

Grootkerk-Tax MG, Soussan AA, de Haas M, Maaskant-van Wijk PA, van der Schoot CE. Evaluation of prenatal RHD typing strategies on cell-free fetal DNA from maternal plasma. *Transfusion* 2006; 46:2142-8.

Grootkerk-Tax MG, van Wintershoven JD, Ligthart PC, van Rhenen DJ, van der Schoot CE, Maaskant-van Wijk PA. RHD(T201R, F223V) cluster analysis in five different ethnic groups and serologic characterization of a new Ethiopian variant DARE, the DIII type 6, and the RHD(F223V). *Transfusion* 2006; 46:606-15.

Granulocyte activation

Recognition of microbes

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Neutrophils can bind pathogens to their surface either by means of Pattern Recognition Receptors (PRRs) for microbial structures or by complement and Fc receptors that recognize complement fragments and antibodies covering the microbes. We have pursued the research concerning the Toll-like receptor (TLR) family of PRRs in this system. We expressed different mutants of MyD88, one of the central proteins in the TLR signaling cascade, in CD34+ progenitor cells, which were then differentiated *in vitro* to mature neutrophils. MyD88 proved to be important for the signal transduction of some, but not all TLRs, which was unexpected. We were able to confirm these results in the neutrophils from an individual that suffers from IRAK-4 deficiency, a protein that interacts with MyD88 and is pivotal for the MyD88-dependent pathway of TLR signaling.

We are currently studying the expression and function of intracellular pattern recognition receptors, the so-called Nod-like receptor (NLR) family of proteins. We have shown that neutrophils express various representatives of this protein family, either constitutively or in response to pathogens. Some of these NLRs are found exclusively in neutrophils. Knock-down experiments by means of shRNA *in vitro* cultured neutrophils of the different NLRs will be performed to elucidate their role in pathogen recognition and their effects on neutrophil effector functions and life span. This research was carried out in the group of Prof Jurg Tschopp (Lausanne, Switzerland) and is currently ongoing in close collaboration with this group. For efficient uptake into phagocytic cells, most micro-organisms need to be covered with antibodies and/or complement components, a process called opsonization. In this context we study the importance of mannose-binding lectin (MBL) in the opsonization of various pathogens (bacteria, yeast and fungal species). To determine complement activation and phagocytosis that is dependent on the lectin-pathway, various pathogens such as *S. aureus*, *E. coli*, *Str. pneumoniae*, *C. albicans*, and zymosan, were opsonized with MBL-sufficient, MBL-deficient and other complement factor deficient sera. Readout of the assay was phagocytosis of the opsonized pathogens by neutrophils as analyzed by flow cytometry. Only for the two yeasts

analyzed the opsonophagocytosis was significantly reduced after opsonization with the MBL-deficient sera compared to the sufficient sera. After optimizing the flow cytometry assay for complement deposition, we are now able to measure C3b binding to all pathogens. With lysates of the opsonized micro-organisms, Western blots are currently performed to determine the deposition of MBL, MASP1, MASP2, C3bi, C4, ficolins, IgG and IgM. Preliminary data indicate that complement binding is not always predictive for efficiency of phagocytosis.

We are also involved in several clinical studies regarding MBL-deficiency and susceptibility for immunology-related disease manifestations, such as fever in the neonatal period, juvenile rheumatoid arthritis, cystic fibrosis, and fever in oncology patients after chemotherapy and/or irradiation. In these groups we measured MBL concentrations with a solid-phase ELISA and 6 single nucleotide polymorphisms (SNPs) in the MBL gene by Taqman allelic discrimination. In addition, we have measured MBL levels, zymosan opsonization as well as C3 / C4 activation after mannan binding, in 12 MBL-deficient pediatric oncology patients supplemented with purified MBL during their neutropenic period, in a collaborative phase-II study with the Academic Medical Center in Amsterdam, and the Serum Staten Institute in Copenhagen, Denmark. Twenty study objects have been included and laboratory analysis of all samples has been completed. Statistical analysis of all data is currently ongoing.

Another line of research concerns the impact of Fc γ receptor numbers and structure on the function of neutrophils. Variation in gene copy number (CNP) as well as single nucleotide polymorphisms (SNPs) driven by evolution are regarded as sources for inter-individual differences. We focus on the gene-to-function relationship of low affinity Fc γ receptor gene cluster, notably *FCGR2* and *FCGR3*. By utilizing multiplex ligation-dependent probe amplification (MLPA), we investigate whether variation in the SNPs and CNPs within the *FCGR2* and *FCGR3* gene clusters predisposes to autoimmune diseases such as idiopathic thrombocytopenia (ITP) and Kawasaki Disease (KD). As indicated by the prevalence of an open reading frame (ORF) of *FCGR2C*, Fc γ receptor type IIc is expressed in 18% of healthy individuals, but a significantly higher expression (34%, OR 2.4 (1.3-4.5), $p < 0.01$) was observed in patients with the hematological autoimmune disease idiopathic thrombocytopenia (ITP). Other

experiments have shown that Fc γ RIIc acts as an activating IgG receptor mediating antibody-dependent cellular cytotoxicity by immune cells.

Key publications

Kummer JA, Broekhuizen R, Everett H, Agostini L, Kuijk L, Martinon F, van Bruggen R, Tschopp J. Inflammasome Components NALP 1 and 3 Show Distinct but Separate Expression Profiles in Human Tissues, Suggesting a Site-specific Role in the Inflammatory Response. *J Histochem Cytochem* 2007; 55(5):443-52.

Brouwer N, Dolman KM, van Zwieten R, Nieuwenhuys E, Hart M, Aarden LA, Roos D, Kuijpers TW. Mannan-binding lectin (MBL)-mediated opsonization is enhanced by the alternative pathway amplification loop. *Molecular Immunology* 2006; 43(13):2051-60.

Van Mirre E, Breunis WB, Geissler J, Hack CE, de Boer M, Roos D, Kuijpers TW. Neutrophil responsiveness to IgG determined by fixed ratios of mRNA levels for activating and inhibitory FcRII (CD32): stable over time and unaffected by cytokines. *Blood* 2006; 108:584-90.

NADPH oxidase

Phagocytic leukocytes generate reactive oxygen species as a defense against pathogenic micro-organisms. The enzyme responsible for this reaction is an NADPH oxidase. When phagocytes are activated by binding of opsonized micro-organisms to various surface receptors, the oxidase subunits assemble into an active complex in the plasma membrane of the phagocyte. To analyze this process in detail, fusion proteins of the different oxidase components with various fluorescent protein tags have been stably co-expressed in the macrophage-like cell line PLB-985 by retroviral transduction. These transfectants will be analyzed for fluorescence resonance energy transfer (FRET) on a single-cell level by the group of Dr C Otto (University of Twente) to reveal the relevant protein-protein interactions during oxidase assembly. During the standard screening of neutrophil functions in patients with recurrent infections, we now have found four patients characterized with a novel defect in NADPH oxidase activation. Activation of the oxidase with the bacterial peptide f-Met-Leu-Phe (fMLP) after priming with platelet-activating factor (PAF) or other

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priming agents appeared to be strongly diminished, whereas phorbol myristate acetate (PMA)-induced activation of the oxidase was only slightly affected, and the serum-treated zymosan (STZ) response was unaffected. Every other fMLP-induced response is normal, indicating that the receptor for fMLP is present and functional, and suggesting that the defect is in the signal transduction. The hypothesis that the cells incapable of superoxide production after fMLP stimulation represent not-fully matured neutrophils that are normally not present in the circulation, seems at least partially correct because in these patients a new marker for fully matured neutrophils (EMR3) is significantly lower than in healthy controls.

Mutations in the SBDS gene have been shown to contribute to the Shwachman-Diamond syndrome, which is characterized by neutropenia and defective neutrophil migration. Our studies are aimed to increase the understanding of the cellular and molecular functions of the SBDS protein.

We have shown that SBDS expression levels decrease during neutrophil differentiation in the human promyelocytic PLB cell line and in CD34+ cord blood differentiation cultures. To extend these studies, we are currently studying the effect of SBDS overexpression and knock down on neutrophil differentiation.

Extensive studies investigating SBDS protein expression have revealed that SBDS is expressed in peripheral blood neutrophils, lymphocytes, monocytes and platelets. Interestingly, our immunostainings showed that SBDS protein is enriched in the perinuclear region that co-localizes with the microtubule-organizing center. Moreover, SBDS protein co-localizes with the mitotic spindle during cell division. These data implicate a role for SBDS in the regulation of processes involving the microtubule cytoskeleton, including chromosome segregation during cell division.

Key publications

Kuijpers TW, van Bruggen R, Kamerbeek N, Tool AT, Hicsonmez G, Gurgey A, Karow A, Verhoeven AJ, Seeger K, Sanal O, Niemeyer C, Roos D. Natural history and early diagnosis of LAD-1/variant syndrome. *Blood* 2007; 109(8):3529-37.

Van Manen HJ, van Bruggen R, Roos D, Otto C. Single-cell optical imaging of the phagocyte NADPH oxidase. *Antioxid Redox Signal* 2006; 8(9-10):1509-22.

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Apoptosis

Apoptosis of neutrophils is an important mechanism in the regulation of the duration of an inflammatory response. In the past year we have further evaluated the role of mitochondria in the homeostasis of these cells as compared to other leukocytes. Neutrophils depend mainly on glycolysis for their energy provision, but they do have mitochondria that maintain a membrane potential ($\Delta\psi_m$). $\Delta\psi_m$ is normally generated by the respiratory chain, which is composed of 4 complexes of which I, III and IV have to be organized into supercomplexes to increase the stability of the individual complexes and for efficient transfer of electrons to molecular oxygen and ATP synthesis. We investigated the characteristics of mitochondrial oxidative phosphorylation in human neutrophils as compared to peripheral blood mononuclear cells and HL-60 cells.

Only complex II and complex V displayed similar activity in neutrophil mitochondria compared to the other cells, while the activity of the other complexes was significantly reduced. However, intact neutrophils responded to inhibition of any complex by producing more lactate. This indicates that electrons derived from glycolysis can be shuttled to the respiratory chain in neutrophils, probably via the glycerol-3-phosphate shuttle, instead of producing lactate. $\Delta\psi_m$ in both neutrophils and peripheral blood mononuclear cells was decreased by inhibition of complex III, confirming that also in neutrophils the respiratory chain is responsible for maintaining $\Delta\psi_m$. Respiratory supercomplexes were lacking in neutrophil mitochondria, while they could be detected in the other cells.

In conclusion, mitochondria in neutrophils maintain $\Delta\psi_m$ by respiration but hardly contribute to ATP generation. This apparent discrepancy can be explained by a decreased expression of complex I, III and IV and a lack of respiratory supercomplex organization in neutrophils.

Key publication

Van Raam BJ, Verhoeven AJ, Kuijpers TW. Mitochondria in neutrophil apoptosis. *Int J Hematol* 2006; 84:199-204.

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Granulocyte transfusions

Transfusion of polymorphonuclear (PMN) concentrates can be a promising tool in addition to pharmacological treatment of neutropenic and immunocompromized patients suffering from life-threatening infections. Donor stimulation with a combination of G-CSF and dexamethasone increases the number of neutrophils, allowing sufficient amounts of cells to be collected for granulocyte transfusion therapy. We showed that G-CSF/dexamethasone treatment, followed by irradiation of concentrates has no impact on neutrophil functions, although prolonged survival and phenotypic changes were observed. We are currently investigating the influence of G-CSF/dexamethasone stimulation of the donors on gene expression in neutrophils. To clarify that, RNA has been isolated from neutrophils obtained before and after stimulation with combination of G-CSF and dexamethasone (*in vivo* and *in vitro*), and microarray analysis has been performed. Detailed analysis of gene expression profiles is currently ongoing.

The life-span of neutrophils obtained from donors was prolonged in *in vitro* cultures, which might allow prolonged storage of the product. We showed that 24 h storage of granulocyte concentrates at room temperature results in an almost unchanged product. Currently, the possibility of storage for 48h is being investigated.

Signaling in transendothelial migration

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Epac1-Rap1 signaling

Epac1-Rap1 signaling in cell adhesion and migration

Cyclic AMP is a well established second messenger known to regulate a large variety of cellular processes, including cell proliferation, differentiation, apoptosis, gene transcription, cell adhesion and migration. cAMP signals through its classical effector PKA and through the Rap1 exchange factor Epac1. We found that, in contrast to previous reports, Epac1 protein is expressed in human hematopoietic progenitor cells and primary leukocytes, with the exception of neutrophils. We also showed that activation of Epac1 in monocytes results in increased adhesion and chemotaxis and that Epac1 is distributed in a polarized fashion, accumulating in the uropod of a migrating cell. Finally, we showed that serotonin, a well-known neurotransmitter implicated in inflammation induces Epac1-Rap1-mediated monocyte adhesion, polarization and chemotaxis. Since serotonin has been found in atherosclerotic lesions and asthmatic lungs, the Epac1-Rap1 signaling pathway may well represent a promising target for therapy of inflammatory disorders such as atherosclerosis and asthma.

Epac1 and PKA signaling in endothelium

It is generally accepted that cAMP promotes endothelial barrier function. We found that specific activation of either PKA or Epac1 increased endothelial barrier function and promoted endothelial cell migration. In addition, using an Epac1 knock-down strategy, we demonstrated that Epac1 and PKA control the endothelial integrity by two independent signaling pathways. The PKA signaling seems to be more dependent on integrin engagement than Epac1 signaling in the regulation of endothelial cell-cell contact. Thus, endothelial cells use two independent cAMP-induced pathways for control of cell-cell adhesion and migration.

Microtubules and small GTPases in the regulation of epithelial barrier function

In chronic inflammatory disorders such as in the lung, leukocytes not only extravasate from the vasculature across the endothelium, but also continue

their journey and pass polarized epithelium to reach the inflammatory site. We demonstrated that disassembly of microtubules in lung epithelial cells promotes cell-cell adhesion. MT disassembly in lung epithelium resulted in an increase in the levels of E-cadherin- β -catenin complexes at cell-cell junctions. Thus, MT may regulate trafficking of E-cadherin- β -catenin complexes to and from cell-cell junctions whereas in the absence of MT, the internalization of junctional complexes is inhibited. Our findings indicate that epithelial cell-cell adhesion induced by MT disassembly is partially mediated through RhoA and Rho kinase (ROK) signaling and is mimicked by inhibition of the Rac1 GTPase. However, in contrast to MT disassembly, the effect of Rac1 inhibition on epithelial cell-cell adhesion does not appear to depend on RhoA/ROK signaling. These observations indicate that two different mechanisms control the increase in epithelial cell-cell adhesion induced by MT disassembly and Rac1 inhibition.

The interaction of ICAM-1 with Filamin and Caveolin-1

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Leukocytes from the circulation migrate towards sites of injury. An important molecule involved in the migration of leukocytes through the endothelium is ICAM-1. This project investigates the molecular mechanisms behind outside-in signaling of ICAM-1 facilitating migration. We have demonstrated that filamin and caveolin-1 interacts with a biotin-labelled peptide resembling the intracellular tail of ICAM-1 as well as with endogenous ICAM in a multimolecular complex. Fluorescent microscopy has shown that; upon ICAM-1 clustering by antibody crosslinking or incubation of ICAM-1 antibody-coated beads on endothelial cells, ICAM-1 becomes translocated to filamin and caveolin-1 enriched regions. Moreover, filamin and caveolin-1 also localize to cup-like structures that surround adherent anti-ICAM-1 coated-beads resembling the so-called transmigratory cups. We show that siRNA-mediated downregulation of filamin (isoform A) reduces the interaction of caveolin-1 with the cytoplasmic tail of ICAM-1, suggesting that caveolin-1 in the complex is dependent on the actin crosslinker filamin. Furthermore, tyrosine phosphorylation impairs the interaction of ICAM-1 with filamin and caveolin-1.

However, the physiological stimulus responsible for this effect remains to be elucidated. Overall, the multimolecular complex which contains filamin and caveolin downstream of ICAM is a dynamic structure involved in transendothelial migration.

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Regulation of the inflammatory response of endothelial cells by RhoB

Pro-inflammatory mediators such as TNF- α , IL-1 β and the bacterial product lipopolysaccharide (LPS) are released during pathogen infection and chronic inflammation. These mediators activate the inflammatory response of the endothelium by i.e. inducing the expression of leukocyte-adhesion molecules which are required for binding and extravasation of leukocytes. Recently, we found that pro-inflammatory mediators induce upregulation and activation of the small GTPase RhoB. Our results show that RhoB is constitutively synthesized by endothelial cells but has a short half-life, being rapidly degraded by the proteasome. TNF- α induces RhoB protein upregulation through NF κ B-dependent gene transcription leading to an increase of at least two-fold in the amount of RhoB present in the cell. The localization of RhoB in intracellular vesicles in TNF-stimulated cells coincides with previous reports where RhoB was shown to regulate vesicle traffic. We have investigated the role of RhoB in the intracellular transport of the TNF receptor and TNF receptor-dependent signaling by knocking-down RhoB expression in endothelial cells using RhoB specific small interfering RNA (siRNA). After TNF addition, RhoB-deficient cells accumulate TNF receptor in intracellular vesicles. In addition, TNF-induced endothelial cell migration is impaired in RhoB-deficient cells. Thus, RhoB may regulate TNF-induced angiogenesis and wound healing and could be a potential target for the therapeutical treatment of atherosclerosis and other chronic inflammatory disorders.

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Molecular mechanisms of hematopoietic (stem)cell migration

The chemokine Stromal Cell Derived Factor-1 (SDF-1/CXCL12) and its receptor CXCR4 are critical for homing to the bone marrow of hematopoietic stem cells after transplantation. This chemokine/receptor pair is also involved in the migration of malignant cells and in the tissue infiltration of inflammatory cells. Interestingly, the chemorepellent Slit proteins and Roundabout (Robo) receptors, initially isolated

from the brain, inhibit CXCL12-induced migration of leukocytes. These data make it conceivable that a balance between positive and negative migratory cues regulate the migratory behavior of hematopoietic (stem) cells to and from tissues. Our goal is to 1) unravel the signaling mechanisms involved in CXCL12-induced migration of hematopoietic (stem) cells and 2) establish the mechanisms mediating the apparent negative migratory signal provided by Slit and Robo.

Ad 1) CXCR4 signaling is abrogated as soon as the receptor is endocytosed in response to the signaling cascade initiated after ligand binding and involves G-protein related kinase and beta-arrestins. The C-terminus appears to be a key player in regulating this process. Based on literature we postulated that the C-terminus has at least 3 functional domains. To investigate this, we have developed biotinylated, cell-permeable peptides representing 3 separate, non-overlapping parts of the CXCR4 C-terminus in addition to the full-length CXCR4 C-terminal peptide. Results show that the full-length C-terminus and the separate proximal and middle domain of the CXCR4 C-terminus are involved in CXCL12-induced actin polymerization, in regulating cell surface CXCR4 expression and in chemotaxis. Biochemical experiments revealed that the full-length C-terminus and the middle peptide interact with beta-arrestin. The full-length CXCR4 C-terminus also bound to endogenous Rac1. In addition, using a proteomics-based approach, we found additional proteins, implicated in receptor trafficking, to interact with the proximal and the middle peptide, respectively. Studies on the relevance for CXCR4 function of these proteins are ongoing.

Ad 2) Four Robo homologs and 3 homologs of the Slit protein have been identified in the vertebrate system, however, nothing was known about the expression pattern of the Robo and Slit homologs in the hematopoietic system. Quantitative mRNA and protein expression analysis revealed the expression of Robo1, Robo2, Robo3, as well as one of the ligands, Slit 1, in various hematopoietic cell types. Interestingly, Robo1 mRNA and surface expression levels of the protein were the highest in CD34+ hematopoietic stem cells, as compared to more mature lineages. Robo1 expression was reduced during myeloid differentiation *in vitro*. Furthermore, both

protein and mRNA expression levels of Robo1 protein on hematopoietic cells were downregulated by exposing the cells to the chemokine CXCL12. The functional relevance of Slit/Robo in migration and differentiation of hematopoietic (stem) cells and the underlying molecular mechanisms are currently under investigation.

Rac1-induced cell migration requires membrane recruitment of the nuclear oncogene SET

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The Rho GTPase Rac1 controls cell adhesion and motility. The effector loop of Rac1 mediates interactions with downstream effectors, whereas its C-terminus binds the exchange factor beta-Pix, which mediates Rac1 targeting and activation. We found that Rac1, through its C-terminus, also binds the nuclear oncogene SET/12PP2A, an inhibitor of the serine/threonine phosphatase PP2A. We observed that SET translocates to the plasma membrane in cells that express active Rac1 as well as in migrating cells. Membrane targeting of SET stimulates cell migration in a Rac1-dependent manner. Conversely, reduction of SET expression inhibits Rac1-induced migration, indicating that efficient Rac1 signaling requires membrane recruitment of SET. The recruitment of the SET oncogene to the plasma membrane represents a new feature of Rac1 signaling. Our results suggest a model in which Rac1-stimulated cell motility requires both effector loop-based downstream signaling and recruitment of a signaling amplifier, that is, SET, through the hypervariable C-terminus.

The role of small GTPases and their activators in leukocyte transendothelial migration

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During transendothelial migration, leukocytes use adhesion receptors, such as ICAM1 and VCAM1, to adhere to the endothelium. Subsequently, the endothelium forms 'transmigratory cups', dynamic membrane protrusions that partially surround adherent leukocytes. Uncontrolled regulation of these cups likely affects the migration of leukocytes across the endothelium, resulting in physiological disorders such as chronic inflammation and atherosclerosis. Thus, it is of high importance to understand the details of the formation of these cups. However, little is known about the signaling pathways that regulate these structures. Our work focuses

on the understanding of the formation of 'transmigratory cups'. Preliminary data suggest that endothelial small GTPases, such as Rac1, RhoA and RhoG are activated downstream from adhesion receptors. Moreover, we are studying how these small GTPases are activated. Recent data using biochemical approaches such as pulldown assays indicate the involvement of guanine-nucleotide exchange factors, enzymes that are able to specifically activate small GTPases, in the formation of these cups. Currently, we are using siRNA techniques to reduce the expression of candidate GEFs and small GTPases and studying their effect on leukocyte transendothelial migration. Moreover, these studies will be extended in flow models to mimic physiological conditions in real life. Together our research will define new signaling pathways that are crucial for leukocyte transendothelial migration and may improve the development of new therapies to treat disorders such as chronic inflammation and atherosclerosis.

Key publications

Hordijk PL. Regulation of NADPH Oxidases: the Role of Rac Proteins. *Circulation Res* 2006; 98:453-62.

Ten Klooster JP, Jaffer ZM, Chernoff J, Hordijk PL. Targeting and activation of Rac1 are mediated by the exchange factor β -PIX. *J Cell Biol* 2006; 172:759-69.

Lorenowicz MJ, van Gils J, de Boer J, Hordijk PL, Fernandez-Borja M. Epac1-Rap1 signaling regulates monocyte adhesion and chemotaxis. *J Leukocyte Biol* 2006; 80:1542-52.

Hordijk PL. Endothelial signaling events during leukocyte transmigration. *FEBS Letters* 2006; 273:4408-15.

Hemostasis and thrombosis

Biosynthesis of the factor VIII-von Willebrand factor complex

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Factor VIII and von Willebrand Factor (VWF) circulate in plasma in a non-covalent complex. It is generally assumed that VWF acts as a molecular chaperone that protects factor VIII from proteolytic degradation in the circulation. This is illustrated by the decreased factor VIII levels that are found in plasma of patients with von Willebrand disease variants that fail to interact with factor VIII. In these patients, factor VIII levels are reduced to approximately 20% of that observed in normal plasma, resulting in a mild bleeding tendency. Conversely, elevated levels of VWF have also been linked to increased levels of blood coagulation factor VIII. Elevated levels of factor VIII have been shown to increase the risk for venous thrombosis. This observation underscores the important role for VWF in the regulation of circulating levels of factor VIII. As yet, limited information is available with respect to regulation of plasma levels of VWF. Biosynthesis of VWF occurs in vascular endothelial cells where it 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. Interestingly, the composition of Weibel-Palade bodies can be modified in response to inflammatory stimuli. Upon stimulation of endothelial cells with the cytokine interleukin-1 β , synthesis of the chemotactic cytokine interleukin-8 is upregulated and part of the synthesized IL-8 is co-stored with VWF in Weibel-Palade bodies. Similarly, the chemokine eotaxin-3 is transported to the Weibel-Palade bodies following upregulation of its synthesis by interleukin-4. These observations emphasize the plasticity of Weibel-Palade bodies and point to a crucial role for this subcellular compartment in maintaining vascular homeostasis. Upon stimulation of endothelial cells by agonist such as thrombin or epinephrine, Weibel-Palade bodies undergo exocytosis, resulting in release of surface expression of their contents. We have recently observed that agonists that raise intracellular concentrations of cAMP induce clustering of Weibel-Palade bodies to the microtubule organizing center (MTOC). We hypothesize that clustering of Weibel-Palade bodies following agonist-induced stimulation provides a feed back

mechanism to maintain adequate levels of recruitable hemostatic components and inflammatory mediators. Several findings show that clustering of Weibel-Palade bodies is a tightly regulated process that involves minus end directed transport of these organelles along microtubules. Pharmacological studies suggest that phosphorylation of as yet unidentified targets by protein kinase A controls clustering of Weibel-Palade bodies. The molecular mechanisms that contribute to the clustering of Weibel-Palade bodies are currently explored with emphasis on the regulation of this process by protein kinase A. In parallel, signaling pathways that control exocytosis of Weibel-Palade bodies are currently being dissected. These studies are likely to generate insight into the mechanism that regulate plasma levels of VWF and other Weibel-Palade body components both under steady state conditions and following vascular perturbation.

Key publications

Rondaij MG, Bierings R, Kragt A, Gijzen KA, Sellink E, van Mourik JA, Fernandez-Borja M, Voorberg J. Dynein-Dynactin complex mediates protein kinase A-dependent clustering of Weibel-Palade bodies in endothelial cells. *Arterioscler Thromb Vasc Biol* 2006; 26:49-55.

Rondaij MG, Bierings R, Kragt A, van Mourik JA, Voorberg J. Dynamics and Plasticity of Weibel-Palade Bodies in Endothelial Cells. *Arterioscler Thromb Vasc Biol* 2006; 26:1002-7.

Structure and function of enzyme-cofactor complexes

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The coagulation cascade comprises several serine proteases that act in combination with a non-enzymatic cofactor. During the past 10 years we have been studying the mechanism by which activated factor IX (factor IXa) assembles with its cofactor, factor VIII. The function of factor IXa in the blood coagulation cascade is to activate factor X in a process that requires the presence of phospholipid surface, calcium ions and activated factor VIII (factor VIIIa). Factor IXa alone is a very poor protease that is 'switched on' to a fully active serine protease upon binding to factor VIIIa. The activated cofactor is a heterotrimer composed of A1, A2 and A3-C1-C2 subunits,

and multiple domains contribute to the assembly of the factor X activating complex. We have previously explored which molecular sites are involved in enzyme-cofactor assembly. On both enzyme and cofactor, these sites are overlapping with typical protein-interactive exosites that also bind to other proteins, such as the endocytic receptor low-density lipoprotein receptor-related protein (LRP). This implies that enzyme-cofactor assembly involves an extended binding site of perhaps multiple exosites in order to endow the protease domain with its factor VIIIa-specific 'catalytic switch'. However, the molecular details thereof have remained unresolved so far. In order to address this mechanism we are focusing on the possibility that the non-catalytic domain modules of factor IXa contribute to its catalytic potential. One example is the interconnection between the two EGF-like domains in the factor IXa light chain, which we have previously found to contribute to factor IXa catalytic activity. We now have extended this work by investigating the role of the interface between the EGF2 domain and the protease domain, with particular focus on the putative interdomain contacts in the factor IXa crystal structure. We have constructed mutants wherein such contacts were disrupted by various amino acid substitutions, including N92A, N92H, Y295A and F299A. Recombinant factor IX variants were produced in mammalian cells, and the purified activated variants were analyzed for a variety of functional parameters. None of these substitutions had a major effect on the interaction with small synthetic substrates. All mutants, however, displayed reduced activation of the natural substrate factor X, which proved more prominent in the presence than in the absence of factor VIIIa. Surface Plasmon Resonance studies further revealed that the same substitutions also affect assembly with factor VIII, both with the isolated light chain (domains A3-C1-C2) and the A2 domain of the heavy chain. We conclude that the exposure of interactive sites for factor VIII and factor X involves contacts at the interface of heavy and light chain within the factor IXa molecule. Apparently, the function of factor IXa, and possibly also related serine proteases from the hemostatic system, is highly dependent on interdomain 'cross-talk' between catalytic and non-catalytic modules.

Key publications

Celie PHN, van Stempvoort G, Fribourg C, Schurgers LJ, Lenting PJ, Mertens K. The connecting segment between both epidermal growth factor-like domains in blood coagulation factor IX contributes to stimulation by factor VIIIa and its isolated A2 domain. *J Biol Chem* 2002; 277; 20214-20.

Rohlena J, Kolkman JA, Boertjes RC, Mertens K, Lenting PJ. Residues Phe-342 to Asn-346 of activated coagulation factor IX contribute to the interaction with low-density lipoprotein receptor-related protein. *J Biol Chem* 2003; 278:9394-401.

Fribourg C, Meijer AB, Mertens K. The interface between the EGF2 domain and the protease domain in blood coagulation factor IX contributes to factor VIII binding and factor X activation. *Biochemistry* 2006; 45:10777-85.

Circulating antibodies to blood coagulation factors

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Hemophilia is an X-linked bleeding disorder that is 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 hemophilia B. Inhibitor development renders patients unresponsive to coagulation factor replacement therapy. Mechanisms underlying inhibitor development in hemophilia A have been extensively studied using mouse models for hemophilia A. Two strains of hemophilic mice were generated by targeted insertion of a neomycine cassette into exon 16 (E-16KO) or exon 17 (E-17KO) of the murine factor VIII gene. Both mice strains have less than 1% of circulating factor VIII. Following repeated injections of human factor VIII, these mice develop high titer inhibitors that resemble factor VIII inhibitors that develop in patients with hemophilia A. The generated models have proven useful for studies directed at inhibitor development. However, the current models cannot be used for studies directed at loss of tolerance in

hemophilia A since all mice injected with factor VIII do develop inhibitory antibodies. We have generated transgenic mice expressing human factor VIII cDNA harbouring an Arg593 to Cys substitution, under the control of a liver specific mouse albumin enhancer/promoter. Transgenic huFVIII-R593C mice were crossed with E-16KO mice and immune responses to administered factor VIII were determined with reference to E-16KO mice. No formation of inhibitory antibodies was observed in huFVIII-R593C/E-16KO mice whereas E-16KO mice readily developed inhibitory antibodies following intravenous administration of FVIII. No factor VIII specific memory B cells or antibody secreting cells were found in the spleen and bone marrow of huFVIII-R593C/E-16KO mice. Also, no factor VIII-specific T cell responses were found in splenocytes of huFVIII-R593C/E-16KO mice. These data show that huFVIII-R593C mice are tolerant to intravenously administered factor VIII. We anticipate that this novel model for hemophilia A may prove useful to identify genetic and non-genetic triggers that induce loss of tolerance in hemophilia A.

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. We have isolated a panel of human monoclonal antibodies from the immunoglobulin repertoire of a patient with acquired TTP. Seven different human monoclonal antibodies were isolated which were divided into 4 groups. Six out of 7 human antibodies reacted with the spacer domain of ADAMTS13. The observed epitope specificity is in agreement with previous findings that showed that the spacer domain harbours a major binding site for anti-ADAMTS13 antibodies. A single antibody interacted with the disintegrin-TSR1 domains of ADAMTS13. One of the isolated antibodies, clone I-9, was converted to full-length IgG1. Biochemical analysis revealed that I-9 inhibited the VWF processing activity of ADAMTS13. IgG derived of plasma of patients with

acquired TTP competed for binding of I-9 to ADAMTS13, suggesting that antibodies with a similar epitope-specificity as I-9 are present in plasma of the majority of patients with acquired TTP. Based on these results, we propose that human monoclonal antibody I-9 is representative for pathogenic anti-ADAMTS13 antibodies present in plasma of patients with acquired TTP.

A novel area of interest is antibodies against β 2-glycoprotein I. This is a plasma protein that is involved in the antiphospholipid syndrome. Patients suffering from this syndrome have a history of vascular thrombosis and/or specific pregnancy morbidity. These symptoms are thought to be caused by autoantibodies called antiphospholipid antibodies, although not primarily directed against phospholipids. Several proteins have been described to serve as antigen for antiphospholipid antibodies. It is generally accepted that antibodies recognizing β 2-glycoprotein I are clinically most relevant, especially those anti- β 2-glycoprotein I antibodies with so-called lupus anticoagulant (LAC) activity. Many studies have been performed in search of a pathogenic mechanism by which antiphospholipid antibodies cause thrombosis and/or pregnancy morbidity. Despite the fact that venous thrombosis is the number one clinical symptom in the antiphospholipid syndrome, most groups published data in favour of a pathogenic mechanism in which cells and receptors are involved. This might explain the occurrence of arterial thrombosis, but venous thrombosis is mostly related to alterations in the coagulation system, shifting the hemostatic balance to a more prothrombotic state. We investigated this possibility and found that especially those antibodies that recognize β 2-glycoprotein I and highly correlate with thrombosis display increased resistance against activated protein C (APC-resistance). This APC-resistance differs from the 'classical' APC resistance in that it is not related to the mutation at position 506 in factor V. These findings suggest that increased APC-resistance caused by antiphospholipid antibodies attributes to the prothrombotic phenotype of patients suffering from the antiphospholipid syndrome. This opens the possibility that antibodies against β 2-glycoprotein I contribute to the high incidence of thrombosis *in vivo*.

Key publications

Van den Brink EN, Bril WS, Turenhout EAM, Zuurveld MG, Bovenschen N, Peters M, Yee TT, Mertens K, Lewis DA, Ortel TL, Lollar P, Scandella D and Voorberg J. Two classes of germline genes both derived from the VH1 family direct the formation of human antibodies that recognize distinct antigenic sites in the C2 domain of factor VIII. *Blood* 2002; 99:2828-34.

Bril WS, van Helden PW, Hausl C, Zuurveld MG, Ahmad RU, Hollestelle MJ, Reitsma PH, Fijnvandraat K, van Lier RA, Schwarz HP, Mertens K, Reipert BM and Voorberg J. Tolerance to factor VIII in a transgenic mouse expressing human factor VIII cDNA carrying an Arg⁵⁹³ to Cys substitution. *Thromb Haemost* 2006; 95:341-7.

Luken BM, Turenhout EAM, Hulstein JJ, van Mourik JA, Fijnheer R and Voorberg J. The spacer domain of ADAMTS13 contains a major binding site for antibodies in patients with thrombotic thrombocytopenic purpura. *Thromb Haemost* 2005; 93:267-74.

Luken BM, Kaijen PH, Turenhout EA, Kremer Hovinga JA, van Mourik JA, Fijnheer R, Voorberg J. Multiple B-cell clones producing antibodies directed to the spacer and disintegrin/thrombospondin type-1 repeat 1 (TSP1) of ADAMTS13 in a patient with acquired thrombotic thrombocytopenic purpura. *J Thromb Haemost* 2006; 4:2355-64.

Cellular receptors involved in clearance of factor VIII

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The identification of mechanisms involved in the clearance of factor VIII remains a continuous challenge. Dysfunction of these mechanisms may cause elevated or reduced factor VIII levels, and thus disturb the hemostatic balance. Moreover, knowledge on the clearance mechanism could provide the basis for prolonging factor VIII half-life, which could be beneficial for factor VIII replacement therapy of patients with hemophilia A. In 1999 we and others established that factor VIII binds to the low-density lipoprotein receptor-related protein (LRP). This receptor is a member of the LDL-receptor family, which is involved in the binding and

cellular uptake of a variety of ligands. Since then we have explored the assembly of factor VIII with several other LDL-receptor family members, including very-low density lipoprotein receptor (VLDLR), low density lipoprotein receptor (LDLR) and megalin (LRP2). *In vitro*, these receptors are similar to LRP in that they all exhibit substantial binding to factor VIII. Recently, we have been able to assess the *in vivo* relevance of these interactions using mice with multiple receptor deficiencies. By this approach we demonstrated that VLDLR, which is present on the endothelial cells of the vascular wall, does not contribute to factor VIII clearance to any appreciable extent. Studies using mice with combined LDLR and hepatic LRP deficiency, however, revealed that LDLR does contribute to factor VIII clearance, in particular in the absence of hepatic LRP. Apparently, the clearance of factor VIII involves a dual receptor mechanism, in which LRP and the much smaller LDLR cooperate in its removal from the circulation.

LRP and LDLR generally bind their ligands by electrostatic interactions with positively-charged patches that are exposed at the ligand surface. The factor VIII molecule comprises several of such areas, both in its heavy chain (the domains A1-A2) and in its light chain (domains A3-C1-C2). We have previously established that the A2 domain contributes to LRP binding. Others, however, reported that LRP binding involves the A2 domain. We therefore investigated the role of the factor VIII heavy chain in LRP-binding in more detail. Our studies revealed that the heavy chain as such does not bind to LRP to any significant extent. Surprisingly, however, binding proved markedly enhanced after proteolytic cleavage of the heavy chain by thrombin. Competition studies using recombinant anti-factor VIII antibody fragments revealed that LRP binding involves residues 484-509 in the A2 domain. This is the same region that has been proposed by others to drive the association of factor VIII with LRP and factor VIII clearance. Because LRP-binding to this site requires prior cleavage of the heavy chain by thrombin, we believe that its role may be limited to the clearance of activated factor VIII. In non-activated factor VIII, however, LRP binding seems exclusively driven by the factor VIII light chain. In view of the notion that von Willebrand factor (VWF) protects factor VIII from premature clearance, it seems likely that part of the putative LRP-interactive sites in factor

VIII are buried in the factor VIII–VWF complex. As we described previously, one LRP-binding region resides in the A3-domain. Using an antibody that inhibits the interaction of factor VIII with both VWF and LRP *in vitro*, we now have identified an additional LRP-binding element, which is located in the C1-domain. We propose that these sites drive the clearance of factor VIII once dissociated from its complex with VWF.

Key publications

Bovenschen N, Mertens K, Hu L, Havekes LM, van Vlijmen BJ. LDL receptor cooperates with LDL receptor-related protein in regulating plasma levels of coagulation factor VIII *in vivo*. *Blood* 2005; 106:906-12.

Bovenschen A, van Stempvoort G, Voorberg J, Mertens K, Meijer AB. Proteolytic cleavage of factor VIII heavy chain is required to expose the binding site for low-density lipoprotein receptor-related protein within the A2 domain. *J Thromb Haemost* 2006; 4:1487-93.

Immunology

Immunoglobulins

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Intravenous immunoglobulin (Ivlg) is being used not only for replacement therapy in patients with antibody deficiency, but also in other conditions such as idiopathic thrombocytopenia, Kawasaki syndrome and Guillain-Barre. In applications other than replacement therapy, the mechanisms of action are largely uncertain. Possibilities are a/o: 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).

Dimers in intravenous immunoglobulin

The stability of the IgG dimers present in Ivlg was investigated by repeated fractionation by size-exclusion chromatography. The results indicated heterogeneity among the dimers: some dimers dissociated rapidly, whereas others were substantially more stable. Biacore analysis of dimer formation using Fab and Fc fragments showed an interaction between Fab fragments and Fc fragments, but not between soluble Fab and coated Fab. Interactions with Fc fragments were investigated in other types of immunoassays. The results confirmed that immunoglobulins of the IgG4 isotype may interact with other immunoglobulins in a way reminiscent of Rheumatoid Factor. In some assays this interaction is restricted to IgG4-IgG4 interactions, in other assay formats IgG4 was found to interact with solid phase- coupled IgG1 as well as with IgG4.

Structural and functional properties of human IgG4

Human IgG4 has been found to exchange half-molecules with other IgG4 in the blood, which usually results in asymmetric antibodies (i.e. with two different antigen-combining sites). Such an exchange reaction is not observed upon mixing IgG4 antibodies in buffer. This suggests that the process, which involves breaking disulphide bonds as well as strong hydrophobic interactions, is catalyzed *in vivo*. In close collaboration with Genmab, two IgG1/IgG4 sets of chimeric mouse/human

monoclonal antibodies to two soluble, non-crossreactive monoclonal antigens have been prepared. Mixtures of these antibodies are being tested for exchange of halfmolecules, both *in vivo* (in a mouse model) and *in vitro*.

Role and specificity of IgM in ischemia-reperfusion

The role of human IgM in ischemia-reperfusion is being investigated in close collaboration with the Dept of Experimental Surgery at the Academic Medical Center (AMC). One of the potential targets of IgM is the phosphorylcholine epitope. For this reason, the activity of mouse and human IgM anti-phosphorylcholine is compared with the activity C-reactive protein, a protein with functional similarity to IgM anti-phosphorylcholine. The experiments are performed in an intestinal mouse model.

Key publications

Aalberse RC, Schuurman J. IgG4 breaking the rules. *Immunology* 2002; 105:9-19.

Diemel RV, ter Hart HG, Derksen GJ, Koenderman AH, Aalberse RC. Characterization of immunoglobulin G fragments in liquid intravenous immunoglobulin products. *Transfusion* 2005; 45(10):1601-9.

Inflammation

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The Inflammation Research group focuses on the classical and lectin pathway of complement activation and on development and clinical evaluation of complement inhibitors. It was found that covalent fixation of activated C4 and C3 to C1q occurred during classical pathway activation and not during activation of other complement pathways. A differential antibody sandwich ELISA was optimized, and levels of these novel activation products were measured in various diseases. Patients with rheumatoid arthritis (RA) were demonstrated to exhibit higher expression levels of these activation parameters and plasma levels appeared to correlate with disease activity. A quantitative assay to measure C4 fixation by the MBL route

was established as well as new methods to measure protein levels and biological activities of C4A and C4B.

Key publications

Wouters D, Wiessenberg HD, Hart M, Bruins P, Voskuyl AE, Daha MR, Hack CE. Complexes between C1q and C3 or C4: novel and specific markers for classical complement pathway activation. *J Immunol Meth* 2005; 298:35-45.

Wouters D, Voskuyl AE, Molenaar ET, Dijkmans BAC, Hack CE. Evaluation of classical complement pathway activation in rheumatoid arthritis. Measurement of C1q-C4 complexes as novel activation products. *Arthritis Rheum* 2006; 54:1143-50.

Immune regulation

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In this research line we focus on the regulation of immunological cascades that involve proteases by protease inhibitors. A major inhibitor of classical complement pathway is C1-inhibitor (C1-Inh), a serine protease inhibitor (serpin). The work on the structure and function of the C1-Inh was continued. Based on previous results of a study of a genetic deficiency of C1-Inh a novel recombinant mutant of C1-Inh was designed, as were mutants that lack any carbohydrate group. These mutants have been successfully expressed in *Pichia pastoris* and were demonstrated to be functionally active. Next, the mutants were purified in large quantities for *in vivo* studies. Currently, we are studying the clearance rate and pharmacokinetics of recombinant C1-Inh infused in rabbits in comparison to plasma purified C1-Inh. In addition, we are investigating the effect of the mutations in the recombinant C1-Inh on the pharmacodynamics of the protein in circulation. Finally, we have started to study whether and by which mechanisms C1-Inh has any cell-protective effects in a model simulates the mechanical damage that venous cells experience when used in cardiac bypass constructions. This part of the project is performed in collaboration with prof H Niessen from the Dept of Pathology at the Vrije Universiteit Medical Center.

Next to the extracellular serpin C1-Inh we continued research on Fahsin, the novel protease inhibitor of human neutrophil elastase, cathepsin G and proteinase 3. We had previously generated recombinant Fahsin in yeast and demonstrated that it was functionally active as a reversible inhibitor of human neutrophil elastase and insensitive to chemical and biological oxidation. In the past year, we optimized the Fahsin production and purification method and adjusted it to suit large scale preparation under cGMP conditions. The batches that were generated were subjected to *in vitro* toxicity studies and were demonstrated to be non-toxic. In addition, we demonstrated that Fahsin is able *in vitro* to inhibit elastase activity in sulcus material obtained from patients that suffer from periodontitis. These data now allow that Fahsin can be used to test *in vivo* efficacy in a clinical setting in periodontitis patients. Finally, we are investigating the function of intracellular serpins by studying the role of granzymes and granzyme-inhibiting serpins in the innate and adaptive immune system. The activity of granzyme B is regulated by the human intracellular serpin SERPINB9. Last year, we demonstrated that mast cells express both granzyme B, perforin and SERPINB9 and that GrB and perforin produced by the mast cell line HMC-1 are active in hemolytic assays. These findings pointed to a novel cytolytic mechanism for human mast cells in host defense and/or tumor rejection. This year, we demonstrated that the different types of mast cells release GrB when triggered with their specific physiological stimulus. Next to GrB, cytotoxic T cells and natural killer cells produce Granzyme A (GrA). GrA seems to induce target cell apoptosis via a different cellular pathway than GrB. We started to generate recombinant GrA in the yeast *Pichia pastoris*. Recombinant GrA could be expressed and purified and was demonstrated to be actively able to cleave a specific substrate. Currently, we are investigating the mechanisms by which GrA induces cell death. Finally, we are exploring if we can identify intracellular GrA substrates as well as GrA inhibitors.

Key publications

Wagenaar-Bos IG, Hack CE. Structure and function of C1-inhibitor. *Immunol Allergy Clin North Am* 2006; 26:615.

Antigen presentation

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Antigen Presentation Research addresses the question how the humoral immune response is regulated by MHC class II-mediated antigen presentation in B cells and dendritic cells. On one hand we continued our research on the regulation and cellular mechanisms that lead to efficient MHC class II molecules in normal and malignant B cells in cooperation with the Depts of Hematology and Pathology of Vrije Universiteit Medical Center. In human B cells, effective class II-Ag presentation depends on MHC class II, but also on HLA-DM (DM) and HLA-DO (DO), the chaperones that regulate the composition of the antigenic peptide repertoire. Last year, we demonstrated that aberrations in the expression of these proteins in malignant B cells correlate with disease status in B cell chronic lymphocytic leukemia (B-CLL). In addition, we have identified aberrancies in the T cell compartment of these patients. This year, we demonstrated that in B-CLL the T cell compartment is shifted from a naive status towards an effector phenotype. This points to an antigen driven process. In addition, we demonstrated that the observed T cell deviations correlate to the previously observed aberrancies in the antigen presentation pathway of the malignant B cells. Next, we have generated B cell line systems expressing the various components of the class II antigen presentation pathway tagged to fluorescent reporters to study the dynamics of B cell-mediated class II antigen presentation in live cells. We demonstrated that particulate antigens and bacteria that are recognized by the B cell receptor (BCR) are rapidly internalized and induce antigen presentation. Thus, B cells behave as professional phagocytes when triggered via their BCR. We are currently investigating the mechanisms of phagocytosis and the consequences for bacterial survival and the generation of a specific humoral immune response. We have continued our collaboration with the Dept of Experimental Immunohematology to develop clinically approved, validated and cost-efficient monocyte-derived dendritic cell products, which will serve as product in clinical trials for tumor vaccination and possibly tolerizing therapy in autoimmune disease and transplantation. Last year, we set up the methods to generate both immature and mature DCs from monocytes and the assays to monitor DC effector function. In collaboration with the Blood Bank North West Region we

now developed a leukocytapheresis method using the MCS+ (Haemonetics®) to obtain enough monocytes for DC preparation. Moreover, we are evaluating the use of the Elutra™ for closed system purification of monocytes from the above mentioned leukocytapheresis product and to developing culture conditions of DCs in bags in order to be able to generate DCs in a closed system. We evaluated the effect of the presence of granulocytes in the monocyte preparation on the efficacy of generation of DCs, as granulocytes and monocytes are collected in the same fraction from the Elutra™. We found that presence of 20-30% granulocytes in the monocyte preparation had no major influence on the generation of DCs. As the DCs that are currently applied in the clinics are suboptimal for the induction of effective antitumor response, we developed a new maturation-cocktail via which effective Th1 inducing DCs can be generated (essential for effective anti-tumor responses). The newly matured DCs are still capable to migrate, another important requirement for an effective DC product. A patent application has been filed to protect this finding for future application in cellular immunotherapy programmes of Sanquin. Finally, research on the use of tetrameric MHC class II molecules as tools to monitor antigen specific T cells in relation to antibody formation against therapeutic proteins was continued and we are currently generating a new type of MHC class II tetramers.

Key publications

Haanen JBAG, Baars A, Gomez R, Weder P, Smits M, de Gruijl TD, von Blumberg BME, Bloemena E, Scheper RJ, van Ham SM, Pinedo HM, van den Eertwegh AJM. Melanoma-specific Tumor-Infiltrating Lymphocytes predict survival in vaccinated advanced-stage melanoma patients. *Canc Immunol and Immunother* 2006; 55:451.

Ten Brinke A., Karsten ML, Dieker MC, Zwaginga JJ, Vrieling H, van Ham SM. Generation of dendritic cells for immunotherapy is minimally impaired by granulocytes in the monocyte preparation. *Immunobiology* 2006; 211:633.

Immunomodulation of blood transfusions in transplantation tolerance

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Aim of the study is to define requirements of blood transfusions intended for induction of allogenic tolerance by unraveling the transfusion effect. In particular donor selection and *in vitro* treatment/modification of donor APC's prior to transfusion is central in this study.

In a retrospective study in a group of kidney-pancreas transplant patients (1996-2006), we observed that administration of pre-transplantation one-HLA-DR shared blood transfusions resulted in significant less severe acute rejection episodes, necessitating ATG treatment, compared to patients without a protocolled blood transfusion.

This effect was irrespective of induction therapy with either ATG or daclizumab, which suggests that a pretransplant protocolled blood transfusion is still valuable combined with current post-transplant immunosuppressive drug therapy. Article is ready for submitting.

In 2004 a prospective study started in recipients of combined kidney-pancreas transplantation. Patients deliberately receive a 1 HLA-DR shared red blood cell concentrate with buffy-coat. Currently from 30 prospective kidney-pancreas patients blood is withdrawn prior, and 2 and 10 weeks after transfusion. PBMCs are cryopreserved and will be evaluated for enumeration of Tregs, primary T cell responses, allo-antibodies and donor chimerism.

Because the hypothesis is that Tregs maintaining tolerance are induced by indirect T cell stimulation, a model is sought to measure indirect antigen stimulation *in vitro* using (overlapping) peptides and allogeneic cell lysates. Since we performed different experiments showing that indirect T cell stimulation tests as published in the literature are of questionable quality and lack appropriate controls for irrelevant peptides and direct stimulation. Extensive *in vitro* experiments applying published protocols show that validation of a model to measure indirect allorecognition by T cells is impossible and questions the results of other studies. Collaborations: Prof FHJ Claas, Dr DL Roelen, LUMC Leiden.

Auto-immune Diseases

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The Auto-immune Diseases research group aims to identify mechanisms that underlie the formation of auto-antibodies. The goal is to verify the hypothesis that impaired clearance of apoptotic cells may result from defects in the proteins contributing to the clearance and lead to an increased risk for the formation of auto-antibodies against nuclear antigens which in their turn may lead to systemic lupus erythematosus (SLE). When apoptotic T cells are incubated with plasma or serum a number of plasma proteins bind to late apoptotic cells. As a side effect we noticed that incubation with plasma lead to rapid removal of nucleosomes from the dead cells. In the absence of serum this takes days but with as little as 5% serum the removal of nucleosomes is completed in 30 minutes. In a large percentage of SLE sera this activity seems to be absent. We have now identified the responsible plasma protein and have developed monoclonal antibodies to it. Those antibodies are now used to affinity purify this protein from human plasma and to develop a quantitative elisa. Work on the role of Toll-like receptors in the stimulation of inflammatory cytokines was continued. We identified a serum factor that is instrumental in stimulating IL-8 production in TLR4/CD14 transfected HEK 293 cells. Patients with rheumatoid arthritis (RA) have antibodies to citrullinated proteins. We have set up an assay to measure antibodies to citrullinated human fibrinogen and verified its diagnostic and prognostic value in early arthritis. As model for antibody formation in auto-immune conditions, antibody formation to the TNF inhibitor drugs Infliximab, adalimumab and Enbrel in patients with RA was investigated. Anti-TNF drugs are nowadays a standard treatment of RA, but clinical responses become limited over time in most patients. In cooperation with the Dept of Rheumatology of the Vrije Universiteit Medical Center, the Academic Medical Center, Slotervaart Ziekenhuis and the Jan van Breemen Institute levels of Infliximab and anti-Infliximab antibodies were measured in RA patients. Formation of neutralizing antibodies indeed seems to be a major cause for diminished clinical efficacy.

Key publications

Wolbink GJ, Voskuyl AE, Lems WF, de Groot E, Nurmohamed MT, Tak PP, Dijkmans BA, Aarden L. Relationship between serum trough infliximab levels, pretreatment C reactive protein levels, and clinical response to infliximab treatment in patients with rheumatoid arthritis. *Ann Rheum Dis* 2005; 64:704-7.

Zwart B, Ciurana C, Rensink I, Manoe R, Hack CE, Aarden LA. Complement activation by apoptotic cells occurs predominantly via IgM and is limited to late apoptotic (secondary necrotic) cells. *Autoimmunity* 2004; 37:95-102.

Wolbink GJ, Vis M, Lems W, Voskuyl AE, de Groot E, Nurmohamed MT, Stapel S, Tak PP, Aarden L, Dijkmans B. Development of antiinfliximab antibodies and relationship to clinical response in patients with rheumatoid arthritis. *Arthritis Rheum* 2006; 54:711-5.

Nielen MM, van der Horst AR, van Schaardenburg D, van der Horst-Bruinsma IE, Van de Stadt RJ, Aarden L, Dijkmans BA, Hamann D. Antibodies to citrullinated human fibrinogen (ACF) have diagnostic and prognostic value in early arthritis. *Ann Rheum Dis* 2005; 64:1199-204.

Blood transmitted infections

Virological aspects of AIDS pathogenesis

Increased neutralization sensitivity of recently emerged CXCR4-using human immunodeficiency virus type 1 strains compared to coexisting CCR5-using variants from the same patient

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CXCR4-using (X4) human immunodeficiency virus type 1 (HIV-1) variants evolve from CCR5-using (R5) variants relatively late in the natural course of infection in 50% of HIV-1 subtype B-infected individuals and subsequently coexist with R5 HIV-1 variants. This relatively late appearance of X4 HIV-1 variants is poorly understood. Here we tested the neutralization sensitivity for soluble CD4 (sCD4) and the broadly neutralizing antibodies IgG1b12, 2F5, 4E10, and 2G12 of multiple coexisting clonal R5 and (R5)X4 (combined term for monotropic X4 and dualtropic R5X4 viruses) HIV-1 variants that were obtained at two time points after the first appearance of X4 variants in five participants of the Amsterdam Cohort Studies on HIV-1 infection and AIDS. Recently emerged (R5)X4 viruses were significantly more sensitive to neutralization by the CD4-binding-site-directed agents sCD4 and IgG1b12 than their coexisting R5 viruses. This difference was less pronounced at the later time point. Early (R5)X4 variants from two out of four patients were also highly sensitive to neutralization by autologous serum (50% inhibition at serum dilutions of > 200). Late (R5)X4 viruses from these two patients were neutralized at lower serum dilutions, which suggested escape of X4 variants from humoral immunity. Autologous neutralization of coexisting R5 and (R5)X4 variants was not observed in the other patients. In conclusion, the increased neutralization sensitivity of HIV-1 variants during the transition from CCR5 usage to CXCR4 usage may imply an inhibitory role for humoral immunity in HIV-1 phenotype evolution in some patients, thus potentially contributing to the late emergence of X4 variants.

T cell line passage can select for pre-existing neutralization-sensitive variants from the quasispecies of primary human immunodeficiency virus type-1 isolates

Primary human immunodeficiency type 1 viruses (HIV-1) resist antibody neutralization but become sensitive after passage through T cell lines. We and others previously reported an increased neutralization sensitivity of HIV-1 after

prolonged culture on primary peripheral blood mononuclear cells (PBMC). Hence we hypothesized that adaptation to growth in T cell lines is in fact selection of a pre-existing neutralization-sensitive HIV-1 variant from the quasispecies in the PBMC culture. Indeed, increased neutralization sensitivity was associated with largely identical synonymous and non-synonymous mutations between progeny of parallel H9 passages from the same split inoculum from 2 of 3 viruses. H9 T cell line adaptation of molecular cloned HIV-1 was less successful and associated with only a few de novo mutations that varied between parallel H9-adapted progeny from the same split inoculum. We conclude that T cell line adaptation of HIV-1 can indeed select for a pre-existing variant but that this most likely depends on the viral diversity in the inoculum.

Antiviral activity of HIV type 1 protease inhibitors nelfinavir and indinavir *in vivo* is not influenced by P-glycoprotein activity on CD4+ T cells

P-glycoprotein (P-gp) can compromise the antiretroviral effect of a protease inhibitor (PI)-containing regimen for HIV-1, but can also reduce HIV-1 replication. We studied the net effect of P-gp on the intracellular HIV-1 RNA and DNA load *in vivo*. CD4+ T cells were isolated from 27 HIV-1 patients (13 without and 14 with a PI-containing regimen) and subsequently sorted in CD45RO(-) (naive) and CD45RO+ (memory) subsets with either high (P-gp(high)) or low (P-gp(low)) P-gp activity. Unspliced HIV-1 RNA and HIV-1 DNA load were determined. For each patient P-gp(high) and P-gp(low) subsets were compared. In patients on a PI-containing regimen, intracellular unspliced HIV-1 RNA was significantly lower in P-gp(high)-naive CD4+ cells compared to P-gp(low)-naive CD4+ cells ($p = 0.04$). The same trend was seen in naive CD4+ cells of treatment naive patients. In both treated and untreated patients HIV-1 DNA levels were significantly lower in P-gp(high) than in P-gp(low) memory CD4+ cells ($p = 0.02$ and $p = 0.04$). High cellular P-gp activity coincided with a reduced intracellular HIV-1 load *in vivo*, both in therapy-naive and in PI-treated patients. Therefore we conclude that the potential efflux function of P-gp on PIs may be clinically less relevant than the effect of P-gp on intracellular HIV-1 replication.

Key publications

Sankatsing SU, Cornelissen M, Kloosterboer N, Crommentuyn KM, Bosch TM, Mul FP, Jurriaans S, Huitema AD, Beijnen JH, Lange JM, Prins JM, Schuitemaker H. Antiviral activity of HIV type 1 protease inhibitors nelfinavir and indinavir *in vivo* is not influenced by P-glycoprotein activity on CD4+ T cells. *AIDS Res Hum Retroviruses* 2007; 23(1):19-27.

Bunnik EM, Quakkelaar ED, van Nuenen AC, Boeser-Nunnink B, Schuitemaker H. Increased neutralization sensitivity of recently emerged CXCR4-using human immunodeficiency virus type 1 strains compared to coexisting CCR5-using variants from the same patient. *J Virol* 2007; 81(2):525-31.

Quakkelaar ED, Beaumont T, van Nuenen AC, van Alphen FP, Boeser-Nunnink BD, van 't Wout AB, Schuitemaker H. T cell line passage can select for pre-existing neutralization-sensitive variants from the quasispecies of primary human immunodeficiency virus type-1 isolates. *Virology* 2007; 359(1):92-104.

Evidence for a role of Trim5 α in AIDS pathogenesis

Recently, the tripartite interaction motif 5 α (Trim5 α) has been identified as an inhibitory factor blocking infection of a broad range of retroviruses in a species specific manner. In particular, HIV-1 replication can be efficiently blocked by Trim5 α from Old world monkeys. The viral determinant in HIV-1 for Trim5 α is believed to be the cyclophilin A (CyPA) binding region in capsid, and mutations in this region lift the restriction in simian cells. Human Trim5 α is also able to inhibit HIV-1 replication *in vitro*, implicating that Trim5 α may contribute to host control of HIV-1 replication *in vivo*.

Here we studied the potential role of Trim5 α in HIV-1 pathogenesis using Trim5 α escape mutations as an indicator for Trim5 α mediated inhibition *in vivo*. Trim5 α escape mutants could be demonstrated in 13.7% of the HIV-1 infected individuals, and these variants emerged relatively late in infection after a prolonged asymptomatic phase. Concomitantly, a significant lower plasma viral RNA load was observed 18 months after seroconversion in individuals that developed Trim5 α

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escape variants late in infection as compared to individuals that only carried Trim5 α sensitive variants. Our data suggest a role for Trim5 α in control of viral burden during the asymptomatic phase of HIV-1 infection.

Key publication

Rits MA, van Dort KA, Munk C, Meijer AB, Kootstra NA. Efficient Transduction of Simian Cells by HIV-1-based Lentiviral Vectors that Contain Mutations in the Capsid Protein. *Mol Ther* 2007; 15(5):930-7

Determinants of *in vitro* HIV-1 susceptibility

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A wide range in susceptibility to human immunodeficiency virus type 1 (HIV-1) is seen among individuals. *In vivo* and *in vitro* studies reveal that both viral and host factors are likely to influence this variability. Preliminary data from *in vitro* experiments show more variation in HIV-1 susceptibility between donor monocyte derived macrophages (MDM) than peripheral blood lymphocytes (PBL) after infection with different HIV-1 isolates. Certain donor MDM are susceptible to almost all HIV-1 strains, whereas other donor MDM are hardly or not susceptible to any HIV-1 strains. The majority of underlying mechanisms responsible for this huge variation in HIV-1 susceptibility between donors remain unknown. By determining *in vitro* HIV-1 susceptibility of uninfected donors followed by genome wide single nucleotide polymorphisms (SNP) genotyping, we want to characterize additional host factors that contribute to HIV-1 susceptibility *in vivo*. This study is focused on the development of a robust assay for donor phenotyping using donor MDM by studying the optimal experimental setup, robustness, and comparing infections of several macrophagetropic viruses such as the virus isolate HIV-1_{BAL} and the molecular clones pYU2, pNL4-3_{BAL}, and pYK-JRCFSF.

To establish this we infected donor MDM and PBL with a range of 33pg – 33ng of viral gag p24 levels in either 24-well or 96 well plates and collected culture supernatants at day 7, 14, and 21. Cultures in 96-well plates gave more robust results, are less laborious and less costly. Many donors remained negative for virus production after infections of donor MDM with pNL4-3_{BAL} and pYK-JRCFSF. In contrast, infections with HIV-1_{BAL} and pYU2 resulted in over 3 log₁₀ variability in

HIV-1 susceptibility between donors, especially with inputs of 1000pg and 3300pg p24, respectively. Furthermore, donor rankings revealed that collecting culture supernatants after these virus infections was most reliable and reproducible at day 14 for HIV-1_{BAL} and at day 7 for pYU2. In addition, we determined that the differences in virus replication between the 4 viruses were probably due differences in the ratio between infectious particles and p24 levels rather than intrinsic differences between these 4 viruses in their ability to infect MDM. Given that pYU2 easily yields high virus titer stocks, we prefer to use this virus. For most donors we also determined HIV-1 susceptibility of their peripheral blood lymphocytes (PBL) after PHA-stimulation. Remarkably, in 2 of 11 experiments with HIV-1_{BAL} and 1 of 13 experiments with pYU2, donors ranked highly susceptible (top 25%) for one cell type were ranked hardly susceptible (bottom 25%) for the other cell type. This suggests that certain host factors, may only play a role in certain cell types and that by studying both cell types more host factors relevant to HIV-1 infection may be uncovered.

Transfusion Technology Assessment

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Notwithstanding that blood products for transfusion are very safe, with new technologies there are always means to improve safety even further. As blood is an allogeneous material of humans who are exposed to a changing environment with new threats, complete blood safety can never be obtained. In addition emerging infectious diseases may require new safety interventions. Dutch governmental policy is to balance such developments aiming at 'optimal blood safety' versus what is considered as 'maximum blood safety'. Optimal blood safety however is still not well defined, and cost-utility or cost-effectiveness analysis of safety measures are to be included in the decision process. Risk assessments underlying these analyzes draw attention not only to the decision models but also to communication of present risk – or safety – level to regulatory bodies and the public. With increasing pressure on cost containment in health care, cost-effectiveness analyzes of blood safety interventions are internationally become more relevant. For these analyzes,

nationally representative data on clinical blood use and blood recipient profiles, including recipient survival, are needed in addition to risk analyzes of adverse outcomes of blood transfusion.

Given a new emerging infectious disease, the risk of disease or negative health outcome for recipients of different blood products needs to be assessed. Such assessments require modeling of the transfusion chain as well as costs and effects of given interventions: properties of the (new) infectious agent, donor epidemiology and donation behavior, test characteristics, processing and inactivation steps and distribution of the end products.

In collaboration with the Dept of Medical Technology Assessment of the Julius Center for Health Sciences and Primary Care at the Utrecht University, a 'Transfusion Technology Assessment' group has been formed, with the explicit mission to perform risk assessments and cost-effectiveness analyzes on blood safety and to establish a nationally representative database of clinical blood use and blood recipient profiles. This means measurement and modeling of costs and effects associated with emerging threats given the national blood transfusion data and the evaluation of proposed blood safety interventions.

Ten years of blood transfusions: use, disease and survival (PROTON-study)

In the Netherlands about 954,500 blood component transfusions are given annually. As Sanquin and hospitals are separate organizations, and hospitals have diverse information systems, little quantitative information on transfusion recipient profiles is known. The distribution of various patient groups, underlying diseases, the amount and type(s) of components transfused, and the survival of the recipients are parameters required for evaluating the (cost-) effectiveness of blood safety interventions. All recorded transfusions in the University Medical Hospital Utrecht (UMCU) from 1993 to 2003 were collected. Transfusion records were linked to general hospital patient records and subsequently anonymously linked to the national decedents register at the National Bureau of Statistics Netherlands. Permission of the ethical committee was obtained. Recipient survival in terms of blood use and underlying disease was analyzed. Censuring the survival rates proved to be

complex and we asked the Dept of Applied Mathematics of the Technical University Delft for assistance. In the study period 33,000 UMCU patients received 415,000 blood component transfusions. The distributions of number of products used per patient are highly skewed. Most transfusions took place around age 68 for females and age 73 for males. However neonates have the highest transfusion intensity. A highly significant increase in mortality rate with increased number of components transfused is found after all transfusions. After 10-years survival there is still an increase in mortality rate of transfusion recipients as compared to the general Dutch population. The initial study included data from one academic hospital, and does not represent the distributions of blood component transfusions in The Netherlands as a whole. The TTA group now extended collaborations and works together with 4 academic and 16 non-academic hospitals in the PROTON study (PROfielen Transfusie ONtvangers; Profiles Transfusion Recipients). In total around 20 hospitals will be included in the study, randomly selected among Dutch hospitals according to categories of blood use patterns. The blood use in a representative sample of hospitals is modelled, in addition outliers in blood use are included. The hospitals have been contacted and are in the process of data delivery. The TTA researchers wish to express their gratitude for the enthusiastic collaboration of the hospitals.

Viral risks of plasma-derived medicinal products

New European legislation (EMA guideline CPMP/BWP/5180/03) requires a viral risk assessment for HBV, HCV, HIV, Parvo B19 and HAV for all new market applications of plasma products. A risk model was developed for Sanquin Plasma Products on the basis of viral and test characteristics, donor epidemiology and Sanquin Virus Safety Studies (VSS) inactivation data. The model has been discussed at confidential meetings of the International Plasma Fractionation Association (IPFA) with risk assessors of the Biotechnology Products Laboratory (BPL) of the United Kingdom. The results of model sensitivity analyzes show that the residual risk is mainly determined by the viral incidence rate, screening test sensitivity, viral reduction capacity and the product yield. The production pool size and type of donation (apheresis or whole blood donation) have low impact on the residual risk. Increasing the inventory hold period has a modest impact on the residual risk,

only 0.5 logs for 1 year increase in hold period. The results show that there is large dispersion in the residual risk estimates (2 to 6 logs) depending on type of virus. Monte-Carlo (probabilistic) simulations are essential when estimating residual risks of blood products. This approach in contrast to traditional risk estimation allows incorporation of complex process specific decision strategies into the risk model. It also allows modeling of uncertain model parameters, like incubation time, duration of the window phase or viral load of an infected donation. Counter-intuitive findings were that production pool size and type of donation e.g. apheresis or whole blood donation have a limited impact on the residual risk. The detailed results of the study remain proprietary of Sanquin, and are to be submitted to the European Medicines Evaluation Agency (EMA), however the methodology of the risk assessment, which is first in this field has been presented at conferences and is submitted for publication.

Experimental design and analysis of Viral Validation Studies

A study is performed to evaluate the effectiveness of the design and analysis of the robustness of virus validation studies. The aim is to improve on the output of these expensive experiments through an evaluation of the current process and advanced mathematical modeling and analysis techniques. This work is performed in collaboration with the Dept of Applied Mathematics of the Technical University Delft.

vCJD risk of plasma-derived medicinal products

A risk model was developed for Sanquin on the basis of expert opinion on variant Creutzfeldt-Jakob Disease (vCJD) and estimates on donor epidemiology and production process inactivation data. Monte-Carlo simulations were used for estimating the contamination risk of blood components and plasma products. Model outcomes have been discussed at confidential meetings with the Medical Advisory Board of Sanquin. The results of the study remain proprietary of Sanquin. The methodology has been discussed at the international risk assessment meetings of the International Expert Advisory Group of Health Canada workshops on Iterative Risk Assessment Processes for Policy Development Under Conditions of Uncertainty and Emerging Infectious Diseases.

Monitoring of viral infection incidence rates among blood donors in the Netherlands

Presently EMEA requires manufacturers of plasma products to report on the prevalence and incidence rates of HIV, HBV and HCV in donor populations. A proprietary report is written for Sanquin. An important measure of residual blood safety is the incidence rate. The goal of the project is to develop a monitoring tool, enabling the detection of significant deviations in incidence rates in repeat tested donors. First a developed monitoring tool is used to check changes in the incidence rates on the national level. Second, incidence rates variability is explored and, regional differences within the Netherlands are examined. Furthermore, statistical tests are used to evaluate and thus enable controlling number of the infection rates in the repeat tested donor population. For this purpose, two tests have been developed. As there is a strong dependency between donation frequency of the infected donors and the estimated incidence rate an improved estimation process is performed. To this end the correction of the observed inter-donation interval is made. Advice has been provided on adjusting the present reporting on confirmed positive donors (Van de Bij et al, Transfusion 2006). The report remains proprietary of Sanquin and the results will be discussed at EMEA.

Cost-effectiveness of NAT testing in The Netherlands

Sanquin is to replace its HIV / HCV NAT testing program in 2008, as NAT laboratories will be further centralized and technology needs replacement. In addition NAT for Parvovirus B19 is performed for plasma products and NAT for HBV and HAV is considered. Based on the data of the infectious disease epidemiology among donors of Sanquin, the different NAT test characteristics available for donor screening (especially for HBV) and the recipient survival characteristics of the TTA transfusion chain model, the (incremental) cost-effectiveness ratio's (CER) of the different NAT options will be estimated with priority on the decisions regarding addition of HBV-NAT to the test algorithms. For the modeling the spread of HBV after transmission by transfusion, collaboration with the National Institute for Public Health and Environment (RIVM) has been obtained.

Key publications

Van der Bij AK, Coutinho RA, van der Poel CL. Surveillance of risk profiles among new and repeat blood donors with transfusion-transmissible infections from 1995 through 2003 in the Netherlands. *Transfusion* 2006; 46(10):1729-36.

Janssen MP, van der Poel CL, Buskens E, Bonneux L, Bonsel GJ, van Hout BA. Costs and benefits of bacterial culturing and pathogen reduction in the Netherlands. *Transfusion* 2006; 46(6):956-65.

Quality, safety and efficiency

Pathogen detection and inactivation

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Pathogen inactivation in platelet concentrates by UV-C light

In this project, we study the possibility to use UV-C illumination (as delivered by a lab scale apparatus developed by CAF/DCF Brussels) for pathogen inactivation in platelet concentrates. In this apparatus, various experimental parameters were investigated for their effect on virus inactivation and platelet quality. The amount of residual plasma was found to be the most important factor, with opposite effects on viral kill and platelet quality. The best balance between these opposite effects were obtained by having 10% residual plasma, which can be achieved in blood bank practice by adjustment of the centrifugation procedure of the starting material, i.e. whole blood derived buffy coats.

During the last year we further characterized the main side effect on platelets, i.e. aggregation. At doses of UV-C needed to obtain significant virus kill either in the presence of 10 or 30% plasma, a significant decrease in platelet count was found. With higher doses (between 500 and 1000 J/m²), visible aggregates were formed within 2h after illumination. By using inhibitors of the fibrinogen receptor IIb/IIIa we have now firmly demonstrated that the decrease in platelet count observed at higher dosages of UV-C is due to activation of this receptor, leading to fibrinogen binding and platelet aggregation. Based on published literature and on clinical experiences with these inhibitors, it should be possible to derive a protocol in which these inhibitors are applied to whole human platelet concentrates. Recently, we have characterized the mechanism underlying IIb/IIIa activation and noted that it is entirely different from classical activation routes used by e.g. thrombin. The possibility that the IIb/IIIa complex itself might be affected by UV-C treatment, hence without the involvement of signaling cascades, is now explored in CHO cells transfected with human IIb/IIIa (gift from Prof M Ginsberg, La Jolla, USA).

Key publication

Li J, de Korte D, Woolum MD, Ruane PH, Keil SD, Lockerbie O, McLean R, Goodrich RP. Pathogen reduction of buffy coat platelet concentrates using riboflavin and light: comparisons with pathogenreduction technology-treated apheresis platelet products. *Vox Sang* 2004; 87:82-90.

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Prion detection system for use in validation studies

The introduction of donor screening, testing of donations for viral markers and the use of viral inactivation and elimination steps, have greatly improved the safety records of plasma derived products. Of concern are newly emerging infectious diseases for which such systems are not yet fully implemented, such as prion diseases, for which the best known human disease is Creutzfeldt-Jakob Disease (CJD) and its variant form (vCJD). The goal of our research is to develop an *in vitro* model system to evaluate the potential of various manufacturing steps used in the production of plasma derived products to remove prions.

Scrapie prion positive and negative cell lines were cultured to establish validated mycoplasma-negative cell stocks. Culture conditions were further optimized with respect to passaging, culture media, normal or low-Ig FCS, with or without 5% CO₂, etc. After optimization, conditions for harvesting cell lysates were established. Meanwhile, the prion specific Eliblot assay was used to test cell lysates from both PrPSc positive and negative cells before and after digestion with protease K to distinguish between normal (PrPc) and abnormal (PrPSc) prion proteins. Conditions were found where no Eliblot signal was obtained with the PrPSc negative lysates and good Eliblot signal was obtained with the PrPSc positive lysates. However, upon dilution of the PrPSc positive lysates, it was found that the PrPSc titer was rather low, not sufficient to serve as spiking material in validation studies (need at least 5 logs). Several steps were undertaken to increase the PrPSc titer (include use of only low passage cells, increasing the number of cells per lysate, trypsin and DNase treatment, extensive sonication, increasing the amount of lysate tested, and buffer and incubation conditions of the Eliblot). The culture supernatant contained only very low amounts of abnormal prion protein and therefore was not suitable as a source of low grade aggregate prion material.

Alternatively, the detection limit could be improved if the PrPSc signal could be amplified by *in vitro* cell culture. Therefore, we set up a 96-well infection system to amplify PrPSc obtained from the positive cells by inoculation of the negative cells. Culture, passage and harvest conditions and the minimum number of cells needed

to obtain a positive signal in the Eliblot were determined using the positive cells. Unfortunately, even after 6 passages (3 weeks of culture) no signal amplification could be detected in the inoculated culture.

However, hamster brain homogenate of 263K scrapie infected hamsters resulted in much better titers when tested in the Eliblot. Given that – in the absence of a bioassay - the PrPSc-specific Western Blot is the golden standard in the field, we next set up the Western Blot system for PrPSc. Here, the results were much more promising with titers for the crude 263K hamster brain homogenate as high as 5.6 logs and for the microsomal fraction 6.2 logs (PrPSc Western Blot approximately 100-fold more sensitive than the Eliblot). Also, our PrPSc Western Blot results for a series of samples with known titers from a nanofiltration validation study were in good agreement.

Viral safety of two new Sanquin plasma products

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Producers of plasma derivatives continuously improve the viral safety of their products by, for example, introducing additional virus-reducing steps into the manufacturing process. Here we present virus-elimination studies undertaken for two plasma products, liquid intravenous immunoglobulin (Nanogam) and C1-inhibitor-nf.

Selected process steps were studied with spiking experiments using a range of lipid enveloped (LE) and non-lipid-enveloped (NLE) viruses. The LE viruses used were bovine viral diarrhoea virus (BVDV), human immunodeficiency virus (HIV) and pseudorabies virus (PRV); the NLE viruses used were Parvovirus B19 (B19), canine parvovirus (CPV), hepatitis A virus (HAV) and encephalomyocarditis virus (EMC). After spiking, samples were collected and tested for residual infectivity, and the reduction factors were calculated. For B19, however, removal of B19 DNA was measured, not residual infectivity. To reveal the contribution of viral neutralization, bovine parvovirus (BPV) and HAV were used.

Nanogam: For the combined pH 4.4/15N nanofiltration step, complete reduction (more than 6 log₁₀) was demonstrated for all viruses, including B19, but not for CPV (between 3.4 and 4.2 log₁₀). Robustness studies for this step with CPV showed that pH was the dominant process parameter. Solvent detergent treatment for 10 minutes resulted in complete inactivation (more than 6 log₁₀) of all LE viruses tested. Precipitation of Cohn fraction III resulted in significant removal (3-4 log₁₀) of both LE and NLE viruses. Virus-neutralization assays of final product revealed significant reduction (at least 3 log₁₀) of both BPV and HAV. The overall virus-reducing capacity was more than 15 log₁₀ for LE viruses. For the NLE viruses B19, CPV and EMC, the overall virus-reducing capacities were > 10 log₁₀, > 7 log₁₀, and > 9 log₁₀, respectively. Including the contribution of immune neutralization, the overall virus-reducing capacity for B19 and HAV is estimated to be more than 10 log₁₀.

C1-inhibitor-nf: In the polyethylene glycol 4000 precipitation step an average reduction in infectious titer of 4.5 log₁₀ was obtained for the five viruses tested (BVDV, CPV, HAV, HIV, PRV). Pasteurization resulted in reduction of infectious virus of > 6 log₁₀ for BVDV, HIV, and PRV; for HAV the reduction factor was limited to 2.8 log₁₀ and for CPV it was zero. Fifteen nm filtration reduced the infectious titer of all viruses by more than 4.5 log₁₀. The overall virus reducing capacity was > 16 log₁₀ for the LE viruses. For the NLE viruses CPV and HAV, the overall virus reducing capacities were > 8.7 log₁₀ and > 10.5 log₁₀, respectively. Based on literature and theoretical assumptions, the prion reducing capacity of the C1-inhibitor NF process was estimated to be > 9 log₁₀.

Key publications

Terpstra FG, Parkkinen J, Tolo H, Koenderman AH, ter Hart HG, von Bonsdorff L, Torma E, van Engelenburg FA. Viral safety of Nanogam, a new 15 nm-filtered liquid immunoglobulin product. *Vox Sang* 2006; 90(1):21-32.

Terpstra FG, Kleijn M, Koenderman AH, Over J, van Engelenburg FA, Schuitemaker H, van 't Wout AB. Viral safety of C1-inhibitor NF, *Biologicals* 2007. In press

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Validation of disinfection procedures

Transmission of blood-borne viruses, like human immunodeficiency virus (HIV), hepatitis B virus, and hepatitis C virus (HCV) in clinical and laboratory settings is a major risk concern. Next to preventive measures like wearing protective coats and gloves, cleaning of non-disposable materials and surfaces is essential. In a previous publication (1) it was shown that a high concentration alcohol mixture (80% ethanol and 5% isopropanol) was very effective in reducing lipid-enveloped (LE) viruses like bovine viral diarrhoea virus (BVDV, a specific model for HCV), HIV (a relevant virus), and pseudorabies virus (PRV, a general model for HBV). In a setting where 5% virus was spiked in the alcohol mixture after 20 seconds of treatment already a reduction value of $> 6.0 \log_{10}$ was found. For non-lipid-enveloped (NLE) viruses like canine parvovirus (CPV, a specific model for human parvovirus B19) and hepatitis A virus (HAV, a relevant virus), no reduction for CPV and limited reduction (approximately $2 \log_{10}$) for HAV was found.

In the above experiments, the disinfection procedures were performed in a so-called 'wet setting', i.e. performed in the disinfecting agent. However, in clinical and laboratory settings the viruses also can be dried onto the surface and in this setting the viruses maybe less accessible to the cleaning agents. Moreover, surface-dried viruses may remain infectious and therefore can pose a threat to public health. To help address this issue, we studied 0.1N sodium hydroxide and 0.1% sodium hypochlorite for their capacity to inactivate surface-dried LE (HIV, BVDV, PRV) and NLE (CPV, HAV) viruses in a background of either plasma or culture medium. In addition, 80% ethanol was tested on surface-dried LE viruses. Without treatment, surface-dried LE viruses remained infectious for at least 1 week and NLE viruses even more than 1 month. Irrespective of the disinfectant, inactivation decreased for viruses dried in plasma, which is more representative of viral contaminated blood, than in culture medium. Inactivation by all disinfectants improved when preceded by re-hydration. Interestingly, infectivity of CPV increased after re-hydration and disinfection may thus be overestimated in the absence of re-hydration. This is the first comprehensive study of five important (model) viruses in a surface-dried state

showing persistence of infectivity, resistance to three commonly used disinfectants and restoration of susceptibility after re-hydration. Our results may have implications for hygiene measurements in the prevention of virus transmission.

Key publication

Van Engelenburg FA, Terpstra FG, Schuitemaker H, Moorer WR. The virucidal spectrum of a high concentration alcohol mixture. *J Hosp Infect* 2002; 51:121-5.

Impedance measurement

The project of impedance measurement for detection of bacteria in platelet concentrates granted by the European Commission in the Fifth Framework of demonstration projects has been terminated in October 2006 with a negative result. Despite numerous efforts to improve the sensitivity of the measuring equipment and the sensors to measure impedance changes, the results were not in accordance with the number of bacteria present in platelet concentrates spiked with bacteria. Sanquin Blood Bank North West Region is the administrative coordinator for the Consortium consisting of Magen David Adom blood center in Tel Aviv and a company in Israel, a company in Germany, the Dept of Microbiology of the Slotervaart Hospital and the Blood Bank.

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Improving materials and methods for blood bank processing

Assessment of Compocool WB butane-1,4-diol cooling plates

Collected whole blood is placed immediately under cooling plates filled with butane 1,4 diol to induce a uniform decrease of temperature in all collected units from about 34°C to about 22°C. Hereafter the whole blood units can be stored at ambient temperature until they are centrifuged up to 24 h after collection. New, vertical, butane-1,4-diol plates and special containers with a lid that can be closed have been developed to meet new requirements for transportation and environment. First temperature decrease in a paired experiment comparing

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old and new cooling plates placed in its respective crates has been assessed. The temperature decrease conformed to the requirements. The new plates in the new containers cooled the whole blood units slightly but significantly faster than the old plates. Next whole blood units were separated into blood components following overnight storage with old and new plates. Leuko-reduced red cells in SAGM were prepared and stored for up to 42 days measuring *in vitro* parameters such as blood cell composition, pH, glucose, lactate, ATP and hemolysis. No significant differences were observed between the data of leuko-reduced red cells in SAGM units derived from units of whole blood stored with either cooling plates.

Apheresis monocytes for dendritic cell culturing

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As part of a Sanquin project (principal investigator Jaap Jan Zwaginga MD PhD, Sanquin Research) apheresis techniques were developed to obtain a monocyte-rich component. MCS+ equipment from Haemonetics was used and initially the program to harvest peripheral blood stem cells was applied and fine-tuned to obtain a monocyte-rich blood component in an acceptable collection time. Contamination with granulocytes and platelets preferably should be below 5% each. The product was further processed using the ELUTRA equipment from Gambro to purify and enrich the monocyte fraction. Next the product was handed over to Sanquin Research (Anja ten Brinke, Marieke van Ham) where the cells were cultured to become dendritic cells under sterile and GMP conditions. The first results showed that it is possible to obtain a product with very few granulocytes and a high number of monocytes and further handle it under sterile and GMP conditions. To optimize the procedure time a new apheresis disposable was developed with Haemonetics, however the apheresis product then was too 'clean' for the ELUTRA, i.e. contained too few leukocytes to properly load the ELUTRA and the anticoagulant: blood ratio negatively affected the growth of dendritic cells. Therefore, the apheresis procedure was adjusted until the product data were acceptable for both ELUTRA and culture conditions and next further evaluated for CE marking. With the improved procedure a product containing 0.9×10^9 monocytes and 0.2×10^9 granulocytes could be obtained in about 114 minutes (n=37). The results have been presented on several international meetings, the report has been sent to Haemonetics and will be used for the CE marking application.

Key publication

Ten Brinke A, Karsten ML, Dieker MC, Zwaginga JJ, Vrieling H, van Ham M. Generation of dendritic cells for immunotherapy is minimally impaired by granulocytes in the monocyte preparation. *Immunobiology* 2006; 211(6-8):633-40.

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Apheresis granulocytes from G-CSF stimulated related donors

Request from hospitals have resulted in a protocol to harvest granulocytes via apheresis following G-CSF (without or with hydrocortisone) stimulation of related donors. The blood bank supports and performs the apheresis procedure, which is executed in the hospital under responsibility of the physician in charge of the patient, usually children. In 2005 and 2006, 87 procedures have been performed with the COBE® Spectra™ (GAMBRO BCT) applying the PMN program and with anticoagulant containing 6% hydroxyethyl (HES) starch. The mean volume was 311 mL, with a mean number of granulocytes of 5.0×10^{10} /unit, procedure time on average 114 min. On average every two to three days granulocytes were transfused, for two to three weeks during the aplastic phase of the bone marrow. In most cases this supportive therapy was successful in fighting the (fungal) infections. The 'Northwest/UMCU' granulocyte apheresis protocol and product specifications are now discussed in a national working party consisting of several (pediatric) departments of university hospitals, and apheresis clinical consultants of Sanquin blood banks with the aim to come to national protocols for (related) donor selection and apheresis procedures. This group has submitted a new proposal to Sanquin for further product improvement and validation. The results have been presented on several international meetings and European-wide interest for the protocols was aroused.

Key publication

Sharon RF, Bierings M, Vrieling H, Versluys B, Boelens JJ. Pre-emptive granulocyte transfusions enable allogeneic hematopoietic stem cell transplantation in pediatric patients with chronic infections. *Bone Marrow Transplant* 2006; 37(3):331-3 (letter).

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Development related research program North East Region

In 2006, again various methods and machines were validated and implemented (plasma hemoglobin method, pH Hanna instrument, Bürker red blood cell counting method, coagulation tests using the KC4 coagulation instrument).

For contract research we did three studies testing whole blood filters and three studies testing red cell filters. Furthermore, we tested a new pH measurement device, which make it possible to measure pH of the platelet concentrate in a sterile way without sampling at any moment. The *in vitro* study will continue with an *in vivo* study in 2007.

Besides contract research we did a project in which we tested the platelet capacity of four various platelet concentrate containers. Further, we participated with the *in vitro* test for the HOVON to compare various storage solutions for platelet concentrates. We did a study to find a gold standard for platelet counting using CD41. Also we studied the production of fibrin glue, a new product for Sanquin. The *in vitro* validation has been performed in 2006, but some follow-up studies will be planned in 2007. Also preparations are made to start a multi center study to investigate the effect of fibrin glue in patients that have undergone knee- or hip replacement. One other project was a study for washing of erythrocytes using an automate (APC 215) compared with the present manual method. Only small differences were observed. Two national send arounds were organized by the department, (i) for counting platelets in platelet concentrates and (ii) for counting leukocytes in plasma, red cell concentrates and platelet concentrates. These send arounds will be repeated yearly. A post authorization surveillance (PAS) for monitoring platelet increments after platelet transfusion in three hospitals was finished at the end of 2006. The data of this PAS will be analyzed in 2007.

Key publication

Van der Meer PF, de Wildt-Eggen J. The effect of whole-blood storage time on the number of white cells and platelets in whole blood and in white cell-reduced red cells. *Transfusion* 2006; 46:589-94.

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Quality Assessment and Improvement Program

In 2006 the Quality Assessment and Improvement Program was continued.

The assessment objective is to ensure that:

- (a) the interaction between Blood Bank Divisions (personnel) and customers (donors, hospital laboratories is positive, and
- (b) it promotes good customer service.

The activities of the principal investigator were in 2006 further focused on advisory tasks and assistance in national surveys. On behalf of the Executive Board and Corporate Staff a study was carried out under the complete file of active donors in the Netherlands regarding their general opinion on blood donation (donor satisfaction survey). The results of this study were used in the Donor Service Concept (focused on improvement of donor policy).

A repeat customer satisfaction survey under the hospital laboratories carried out in 2005 was extensively reported in 2006. A planned and extensively prepared survey under the customers of Sanquin ICT was finally postponed. Two studies initiated by Sanquin Blood Bank South East Region were prepared: a second assessment on opinion Blood Bank division internal communication, and opinion of general public and donors concerning possible commercial activities of Eurocord Nederland (ECN).

Improving materials and methods for storage of blood components

Cryopreservation of red blood cells

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Research on the cryopreservation of red blood cells has mainly focused on improving the quality of the red cells during post-thaw storage. The use of a closed system to add and remove glycerol, will allow the storage time after thawing of previously frozen red blood cells to be longer than 24h. With the use of SAG-M as additive solution, hemolysis of thawed cells remained below below 0.8% for 48 hours. AS-3 maintained the integrity of thawed red cells much better than SAG-M: hemolysis remained below 0.8% for 14 days. This difference was explained by the presence

of citrate in AS-3. Under normal conditions, the red cell volume is maintained by a near-impermeability of the RBC membrane to sodium and potassium. The freeze-thaw process disturbs this impermeability of the RBC membrane as can be seen by an increase in extracellular potassium. Due to the presence of hemoglobin, cation-permeable red cells have a greater osmolarity than the external medium. This difference in osmolarity results in water influx, cell swelling and, eventually, hemolysis. With the addition of an impermeant solute, such as citrate, to the additive solution, cell swelling can be prevented.

In contrast to hemolysis, the ATP content of the red cells was better maintained in SAG-M as compared to AS-3. Since there is a correlation between ATP content and *in vivo* survival of red cells, it is important to maintain ATP levels during storage. Cells resuspended in AS-3 have a lower internal pH, which results in a lower glycolytic activity and therefore less production of ATP. Increasing the internal pH of cells before storage in AS-3 by using PBS (pH 7.4) instead of the glucose-containing washing solutions normally used (with a pH of 6.0) in the deglycerolization procedure, resulted in elevated lactate production and better maintenance of intracellular ATP content. Based on the recommendations for hemolysis and RBC ATP concentration, leukoreduced, deglycerolized RBC can be stored for 48 h in SAG-M. When resuspended in AS-3, thawed cells can be stored for 2 weeks at 2-6°C provided PBS is used as washing solution.

Key publication

Lelkens CC, Noorman F, Koning JG, Truijens-de Lange R, Stekinger PS, Bakker JC, Lagerberg JW, Brand A, Verhoeven AJ. Stability after thawing of RBCs frozen with the high- and low-glycerol method. *Transfusion* 2003; 43(2):157-64.

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'Hibernation' project

Glucose deprivation and metabolic suppression by addition of antimycin A can be used to preserve platelet function during prolonged storage at 4°C. However antimycin A cannot be used when the platelets are intended for transfusion to humans, instead, low oxygenation content was applied. In 2005 experiments were performed with platelets in HEPES Tyrode buffer without and with 5 mM glucose in gas impermeable plastic bags to mimic the experiments done in test tubes. Metabolic suppression was observed in platelets stored in the glucose-free medium at 4°C and following 'waking up' with 20 mM glucose TRAP-induced aggregation was recovered, depending on the total storage time at 4°C. To imitate the test tube circumstances platelets were resuspended in the respective media, incubated for 4 h at 22°C to consume the remaining glucose and induce glucose deprivation and next stored at 4°C without agitation in 600 mL bags. When the metabolically suppressed platelets were stored in gas-impermeable bags, the response to recovery with glucose was better than in platelets stored in normal plastic. However these experiments are the first step to translate the laboratory conditions in test tubes to storage of platelets in plastic bags. Further studies are required to prove that this method is suitable for real blood bank conditions; publication has been accepted for publication in *Vox Sanguinis* in 2007. The grant ended in the beginning of 2006, a proposal to continue the study will be submitted in 2007 to Sanquin. The PhD student defended his thesis in 2006. The study was performed in collaboration with the Dept of Thrombosis and Hemostasis of the Utrecht Medical Center (Head Prof JW Akkerman).

Key publications

Badlou BA, Wu YP, Smid WM, Akkerman JW. Platelet binding and phagocytosis by macrophages. *Transfusion* 2006; 46(8):1432-43.

Badlou BA, Wu YP, Spierenburg G, Smid WM, Akkerman JW. Role of glycoprotein Ib-alpha in phagocytosis of platelets by macrophages *Transfusion* 2006; 46(12):2090-9.

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Storage of leuko-reduced red cell concentrates in plastic over-wraps

For many years the Sanquin Blood Bank North West Region has packed leuko-reduced red cell concentrates (LR-RCCs) in a plastic over-wrap as an extra protection during storage and transportation. On request of hospitals LR-RCCs stored at 2-6°C in two types of new bags with a zip lock were compared with LR-RCCs stored in the original bag or stored without an over-wrap. In paired experiments (n=12) the LR-RCCs were sampled at day 28, 35 and 42. Small but significant differences were observed in PO₂; other parameters such as pH, PCO₂, glucose consumption and lactate production and ATP content were comparable. The use of an over-wrap did not affect storage of LR-RCCs. The study was performed on request of the Sanquin Processing Group to enable a final national decision on the use of an over-wrap. A paper has been submitted for publication.

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Erythrocyte storage solutions

During preparation and storage of red cell concentrates (RCC), levels of 2,3-DPG levels fall rapidly. Recently, we developed an additive solution (PA3GM, based on PAGGS-M with saline replaced by gluconate, pH 8.2) allowing maintenance of high 2,3-DPG levels without concurrent ATP decline. Our new solution is based on the 'chloride-shift' principle demonstrated 10 years ago, resulting in a more alkaline cytosol favouring 2,3-DPG formation. Early results showed discrepancies between measured intracellular pH (pHi) and actual effects on 2,3-DPG, which could be explained by assuming memory effects of initial conditions. We therefore investigated the effect of differences in initial conditions on pHi and 2,3-DPG changes during subsequent storage.

The initial pHi of RCC was manipulated by either adding our experimental solution PA3GM during RCC preparation or by washing RCC with the same solution, thereby increasing the extent of chloride depletion. After 21 days of storage at 4°C, the units were split and washed in different solutions. Samples were taken during another 21 days of storage.

The initial pHi of the RCC suspended in plasma/PA3GM was 7.0±0.05 (n=4) and 7.3±0.09 (n=4) with washing in PA3GM. After 21 days, the pHi had decreased to 6.55 for both types of RCC. After washing in PAGGS-M (pH 6.2), pHi in both cases

declined rapidly to 6.0, but the changes in 2,3-DPG were quite different. RCC with an initial pHi of 7.0 showed a fall in 2,3-DPG within 1 week, whereas RCC with an initial pHi of 7.3 showed an increase during 1 week, before the decrease started. Lactate production showed a similar memory for initial pHi. ATP levels remained unchanged upon washing in both cases.

We conclude that changes in the intracellular pH of RCC have long-lasting effects on 2,3-DPG levels and on glycolytic activity. This observation indicates the need not only for other storage media, but also for other collection methods in which a fall in pHi is avoided.

Key publications

De Korte D, Verhoeven AJ. Quality determinants of erythrocyte destined for transfusion. *Cell Mol Biol* 2004; 50:187-95.

Verhoeven AJ, Hilarius PM, Dekkers DW, Lagerberg JW, de Korte D. Prolonged storage of red blood cells affects aminophospholipid translocase activity. *Vox Sang* 2006; 91:244-51.

Improved cryopreservation of hematopoietic progenitor cells: a fundamental approach

Hematopoietic stem cell (HSC) transplants are considered to be the best treatment option for many patients with (hematological) malignancies. In case of autologous transplantation and cord blood transplantation, it is necessary to cryopreserve the stem cells. Although the clinical results show that cryopreservation is successful in maintaining HSC viability, at least to a certain extent, it is generally accepted that cell loss/cell death occurs during cryopreservation. Improved cryopreservation protocols most likely result in decreased cell death and improved engraftment kinetics. This will decrease the costs and morbidity of autologous transplantation. To improve the freezing protocol we used a new theoretical model, developed by Dr H Woelders (Animal Sciences Group, Wageningen UR) for the osmotic events occurring during cryopreservation. This model is mainly based on the permeability of blood cells for water and cryoprotectant. With literature values of cell characteristics and membrane permeability parameters for hematopoietic progenitor

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cells, simulations were performed using various concentrations of DMSO, resulting in predicted optimal freezing protocols. The most striking difference between the predicted freezing protocols and the currently used protocols is the non-linearity of the predicted curve. While the current freezing protocol uses a linear cooling rate, the calculated optimal curves consist of an initial slow cooling rate to -10°C , followed by fast cooling to -35°C , followed by again slow cooling to -50°C after which the cells can be plunged in liquid nitrogen. The optimal cooling rates depend on the concentration of DMSO. The calculated freezing curves for 5% and 10% DMSO were applied in a programmable controlled-rate freezer and used for freezing hematopoietic progenitor cells.

The current method to investigate the quality of HPC, detection of CFU-GM, is very laborious and time consuming. Therefore, a fast and sensitive method to detect cell viability was developed. We used the dye YO-PRO-1 to detect early apoptotic cells in a FACS-based assay. YO-PRO-1 staining is comparable with annexinV staining, but has the great advantage that it can be tested in a No-Lyse No-Wash assay. While living cells are impermeable, YO-PRO-1 can enter early-apoptotic cells. By using YO-PRO-1 in combination with 7-AAD (a vital stain) we are able to make a distinction between living cells (YO-PRO and 7-AAD negative), early-apoptotic cells (YO-PRO positive, 7-AAD negative) and late-apoptotic/necrotic cells (YO-PRO and 7-AAD positive). Initial experiments using this method showed that suspensions frozen with our current freezing protocol (10% DMSO, linear freezing curves) contained high amounts of early apoptotic HPCs. Using a lower DMSO concentration (5%) resulted in increased viability of CD34+ cells. Also the use of a non-linear, freezing curve seemed to increase the viability of the thawed CD34+ cells. In addition, also the formation of CFU-GM and the expansion of megakaryocytes seems to be better when a lower DMSO concentration and/or a non-linear freezing curve is used.

From additional experiments it became clear that besides the freezing curve and the DMSO concentration, also other factors might influence the viability of thawed cells. While several parameters, including cell density and freezer load, could be excluded, it is, at present, not exactly clear which factors, other than the freezing procedure, are involved in the viability of thawed HPCs.

Platelet storage

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Platelet metabolism during storage

Platelets may be stored for 7 days in plasma prior to transfusion. During storage, however, several biochemical parameters change (a phenomenon called 'platelet storage lesion') although the relevance of these changes for *in vivo* functionality is unknown. In our project, the metabolism of platelets during *in vitro* storage is studied. During 2006, the following subjects were studied:

Effect of pH on *in vitro* quality of platelets

The role of pH in relation to the *in vitro* quality of platelet concentrates was elucidated further. The overall conclusion of these experiments is that changes in pH during storage seems to be a result of the so-called 'storage lesion' and not the principal cause for this lesion.

Storage of platelets at 4°C

Because of the renewed interest in possibilities to extend platelet shelf life by storage at 4°C, a pilot study has been performed to establish the status of PCs during storage at 4°C in a mixture of plasma (30%) and PASIIM buffer (70%). During cold storage in PASIIM various *in vitro* parameters remain quite well. The platelets showed low CD62P expression (< 20% positive cells) and PS exposure (< 10%) and the medium still contained 5 mM glucose after 14 days of storage. Aggregation experiments showed that platelets stored at 4°C for up to 14 days, still aggregate upon collagen stimulation, whereas for platelets stored for 7 days at 22°C this response was completely absent. During cold storage, only a minimal decrease of the platelet count was found. Platelets stored at 4°C in PASIIM (with 30% plasma) are reasonably stable for at least 14 days (evaluated with a restricted set of *in vitro* parameters) but should be tested for *in vivo* functionality and survival.

Role of AMP-dependent kinase in platelet apoptosis

Further research on the involvement of metabolic pathways in the maintenance of platelet quality resulted in the following results. We found that uncoupling of

mitochondria in the presence of glucose did not result in increased PS exposure or a lower ATP/ADP ratio. This supported earlier findings that mitochondria play a minor role in providing cytosolic energy.

Based on preliminary results we hypothesized that AMPK activation may be involved in the process of PS exposure (see Scientific report 2005). Extensive experiments were performed to support this notion. This resulted in a highly significant correlation between the phosphorylation status of AMPK and PS exposure. To check whether phosphorylated AMPK is really active, we measured the activity of AMPK by measuring the phosphorylation of Acetyl-CoA carboxylase (ACC) as AMPK substrate, an assay recently introduced in our lab. These experiments showed a good correlation between degree of phosphorylation of AMPK and subsequent phosphorylation of ACC.

The activation of AMPK has been described to involve two kinases, LKB1 and the Calmodulin kinase (CaMK). The inhibition of LKB1 with 5-Iodotubericidin results in partial inhibition of PS exposure. This occurs only under condition of metabolic deprivation. The second route (CaMK) could be responsible for the rest activity causing PS exposure. Therefore several inhibitors of CaMKs were tested, including BAPTA-AM. As CaMK is calcium-dependent, chelation of Ca^{2+} by BAPTA-AM should inhibit CaMK. All inhibitors (W-7, KN62, STO609, BAPTA-AM, Iodotubericidin) were tested in titration experiments (also with combinations). Only for BAPTA-AM treatment in most cases a total inhibition of PS was found. Currently, we are focusing on the activation of platelets by agonists (thrombin and/or collagen) and the role of AMPK on PS exposure during this treatment.

Key publication

Verhoeven AJ, Verhaar R, Gouwerok EG, de Korte D. The mitochondrial membrane potential in human platelets: a sensitive parameter for platelet quality. *Transfusion* 2005; 45(1):82-9.

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Platelet storage in various additive solutions

Platelet concentrates (PCs) are generally stored in plasma up to 7 days provided screening on bacterial contamination is performed. In the recent years a number of platelet additive solutions have been developed and 3 of these Intersol (Baxter), Composol (Fresenius) and SSP+ (Macopharma) were investigated. For one paired experiment 20 buffycoats were pooled and divided into 4 equal portions. To each portion either one of the 3 additive solutions was added or plasma as a reference. All PCs were leuko-reduced (LR) and stored in a 1 L DnDP-PVC storage bag with gentle agitation at 20 – 24°C. Samples were taken aseptically up to day 8 to measure cell content, pH, glucose, lactate, MPV, Annexin V and CD62P. In the LR-PCs stored for 8 days these parameters *in vitro* remained acceptable (n=12 experiments). Of the 3 additive solutions investigated Intersol gave the poorest results. These investigations were part of the national evaluation of storage of LR-PCs for 7 days in additive solutions without and with pathogen reduction. After these investigations *in vitro* a national clinical study will start with increment and bleeding outcomes after transfusion of LR-PCs stored in additive solutions for up to 7 days (HOVON).

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Storage of leuko-reduced apheresis platelets in plasma for pediatric transfusion

The investigations started in 2005, in brief: leuko-reduced apheresis platelets in plasma are divided into 4 units for pediatric transfusion. The whole apheresis unit is stored in a platelet storage bag with a volume between 1 and 1,5 L depending on the manufacturer. The split units are stored in small (600 mL) bags in plasma up to 5 days. On the market are 600 mL bags made from polyolefin, 600 mL bags of another gas permeable material plasticized with butyl-trihexyl-citrate (BTHC) became available. In a paired experiment we investigated pediatric split units of leuko-reduced apheresis platelets stored in plasma up to 7 days in both types of bags. Unfortunately the results were disappointing. The pH and the swirling effect in the bags deteriorated at day 4 to 5 already. The results were summarized and discussed with blood bag manufacturers. In the end of 2006 other bags became available, these will again be investigated.

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Storage of leuko-reduced apheresis platelets in plasma for 7 days

In 2005 the FDA approved storage of leuko-reduced apheresis platelets stored in plasma and collected with the TRIMA apheresis equipment from GAMBRO BCT, provided that this was coupled to a 100% release test screening for bacterial contamination. Storage of whole blood derived LR-PCs in plasma already is 7 days at 20-24°C with gentle agitation, under the same conditions of bacterial screening. Apheresis procedures allow various combinations of equipment, disposables, platelet storage bags, and anticoagulant: blood ratios. The aim of the study was to compare LR apheresis platelets obtained with the TRIMA programmed at 350×10^9 platelets and a concentration of 1×10^9 /mL; the butyl hexyl citrate (BTHC) platelet storage bag is 1.3 L in size. The MCS+ (Haemonetics) was also programmed to obtain 350×10^9 platelets and a maximum concentration of 1.2×10^9 /mL; the BTHC platelet storage bag is 1 L in size. All units were stored at a flat bed shaker in a climate cabinet at 20-24°C and sampled at day 1, 6 and 8 for cell counts, pH, PO₂, PCO₂, glucose, lactate and CD62P. The results showed that the apheresis LR-PCs under the described circumstances met the requirements during 7 days of storage. However, when LR-PCs were harvested with a different program intended to obtain a platelet concentration of 1.5×10^9 /mL and the platelet concentration of the LR-PCs indeed exceeded 1.2×10^9 /mL, acidification occurred and swirling effect disappeared. A paper has been prepared and is submitted for publication.

This assessment underlines the importance of validation when apheresis procedures are changed.

Biochemical and biophysical changes in platelets during storage

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Platelet activation by potent, calcium-mobilizing agonists results in shedding of microparticles (MP) that are active in coagulation. Previously, we have shown that platelets stored in blood bank products, release procoagulant MP in the apparent absence of agonist. Under activation conditions the shedding of MP is mediated by rises in intracellular calcium and calpain activation, leading to protease activity that can degrade the cytoskeleton. However, MP formation by resting platelets, i.e. under blood bank conditions, results from α IIb β 3 outside-in signaling to destabilization of the actin cytoskeleton in the absence of rises in intracellular calcium and calpain activation.

Integrin-mediated spreading of platelets over fibrinogen similarly results in microparticle formation. After transfusion of stored platelet preparations to thrombocytopenic patients, the MPs contribute to coagulant activity *in vivo* within 1 hour after transfusion.

Key publications

Cauwenberghs S, Feijge MA, Harper AG, Sage SO, Curvers J, Heemskerk JW. Shedding of procoagulant microparticles from unstimulated platelets by integrin-mediated destabilization of actin cytoskeleton. *FEBS Lett* 2006; 580(22):5313-20.

Cauwenberghs S, Feijge MA, Theunissen E, Heemskerk JW, van Pampus EC, Curvers J. Novel methodology for assessment of prophylactic platelet transfusion therapy by measuring increased thrombus formation and thrombin generation. *Br J Haematol* 2006; 136(3):480-90.

New functional whole blood assays to assess platelet function under flow

Part of this project aims at determining microparticles (MP) that are released during storage of blood bank platelets. Therefore, we studied whether MP in platelet products i) adhered to collagen, fibrinogen or surface immobilized platelets, ii) supported further platelet deposition and iii) enhanced surface dependent blood coagulation as measured by thrombin-antithrombin (TAT) complexes. To mimic the *in vivo* situation in thrombocytopenic patients experiments were performed in virtual absence of platelets. Thrombocytopenic blood was then supplemented with labelled MP and was perfused through glass capillaries coated with collagen, fibrinogen, vWF or adhered platelets at a low (100/s) or high (1000/s) shear rate. Adhesion was monitored in real-time by fluorescence microscopy. We found that MPs firmly adhere to collagen, VWF, fibrinogen and surface-adherent platelets at low and high shear rate. Antibodies against $Ib\alpha$ and $\alpha IIb\beta 3$ were used to demonstrate the specificities of these interactions. The addition of MPs to the thrombocytopenic blood did not affect platelet adhesion under these conditions. TAT complex formation was increased in the presence of MPs in capillaries coated with fibrinogen, but not on collagen fibers. We confirmed that these storage induced MPs adhere to a damaged vasculature bed *in vivo* after infusion in denuded arteries in a mouse model.

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We thus conclude that MPs in platelet products have platelet-like adhering properties and accelerate thrombin generation. These properties strongly support the notion that MPs can be beneficial in maintaining normal hemostasis when platelet function is impaired or reduced like in thrombocytopenic patients. The thrombin supportive role of MP mainly comes from exposed PtdSer and a positive feedback loop via factor XI activation, however, the anticoagulant properties of MP shed during storage of platelets have not been studied yet.

We investigated the properties of activated factor V (FVa) inactivation on the surface of storage induced MP. We established that MP present in stored platelet products carry FVa at their surface. We have determined the activated protein C (APC) catalysed inactivation of MP-bound FVa. Also, the inactivation of FVa at the surface of thrombin-activated platelets and synthetic vesicles was determined. APC-catalysed inactivation of MP-bound FVa resulted in $42\pm 2\%$ residual FVa activity after 20 min. Residual activity of FVa on thrombin-activated platelets was $25\pm 3\%$. Plasma-derived FVa was rapidly inactivated in the presence of synthetic vesicles, with $5\pm 4\%$ residual FVa activity. When synthetic vesicles were added to the inactivation mixture of MP or thrombin-activated platelets, a residual activity of 5-10% was found. Furthermore, addition of excess plasma-FVa to storage induced MP resulted in a residual activity of $26\pm 2\%$. Moreover, the APC resistant phenotype of MP was confirmed in plasma in which thrombin generation was measured in the absence and presence of APC. Residual FVa activity in the presence of MP, platelets or synthetic vesicles was $87\pm 6\%$, $65\pm 3\%$ and $8\pm 19\%$, respectively.

Together, these results suggest that the MP surface environment renders FVa resistant to APC. We further conclude that the APC resistance of FVa at the surface of storage induced MP enhances their procoagulant nature.

Key publication

Keuren JF, Magdeleyns EJ, Govers-Riemsag JW, Lindhout T, Curvers J. Effects of storage-induced platelet microparticles on the initiation and propagation phase of blood coagulation. *Br J Haematol* 2006; 134(3):307-13.

New therapies and evaluation of clinical applications

New cellular therapies

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Cellular therapy research

Projects in this research line are focused on the development of new cellular therapies. that a) can facilitate the hematopoietic stem cell transplantation and b) can facilitate tissue regeneration.

Mesenchymal stem cells are known in this light to support the efficacy of stem cell transplantation. We hypothesized that MSC have the potential to induce a proangiogenic state of the endothelium. Finding this proangiogenic effects on endothelial cells would explain marrow tissue regeneration and repair and thus enhanced MSC mediated repopulation. Furthermore, using immunohistochemistry of bone marrow sections of NOD/SCID mice, we observed significant differences between irradiated mice which received CD34+ cells and mice which received CD34+ cell in combination with MSC. As part of the Dutch Program on Tissue Engineering we studies the migratory behavior of MSC obtained from different sources.

The other focus of our cellular therapy research deals with the potential of blood or bone marrow derived cells to form the blood vessel lining endothelium. We have previously demonstrated that monocytic cells are responsible for the outgrowth in the so-called Endocult assay, a colony assay for Endothelial colony forming cells (CFU-EC). CD4+ T-cells seemed to facilitate the monocytic colony formation by a still unknown paracrine factor. Direct cell-cell contact was needed for this facilitation. This cell contact could be inhibited by CD3 or MHC-classII antibodies, and could be replaced by activating CD3/CD28 antibodies. These findings suggest that the proangiogenic state of monocytes is mediate by T-cell help in a sterile inflammation reaction. Monocytes activated by T-cells will be tested for their revascularizing potential in the ischemic hind limb model, operational at our department.

We previously showed that stem cells can be expanded *ex vivo* into megakaryocytes by the combination of thrombopoietin (Tpo) and IL-1. These cultures of expanded cells might still be the used to shorten the thrombocytopenic period after autologous stem cell transplantation. In animal studies we have now clearly shown a short term platelet repopulating effect via these expanded stem cell products, and

it was also shown that the CD34+ cells had to be cultured for 7 days. If the cells were cultured for only 4 days, the platelet repopulation was delayed with 3 days. The same cultures were applied to compare the RNA (the transcriptome) of very pure megakaryocytic cultures with that in erythroblast cultures, to define genes that are specifically active in 'blood platelets'. These gene lists are an important basis for a large European Integrated project (Bloodomics), that aims to correlate small variations in those genes to a changed risk for cardiovascular disease. Some of the newly identified platelet specific genes have been studied for their function in platelets. Our department will when promising risk-associated genes are characterized, use so-called gene silencing techniques (siRNA) in megakaryocyte cultures to see what the function is of these often new gene (variations). Despite extensive studies we did not succeed in the *in vitro* production of platelets. We therefore will use the transplantation model of CD34+ cells in NOD/SCID mice to obtain human platelets and test their functionality.

Within the laboratory for Stem Cell Transplantation (SCL) most processes involve the processing of cellular products for conventional autologous and allogeneic transplantation of adults and children. However, in recent years the SCL became closely associated with several clinical trials; at first, the Hebe trial in collaboration with ICIN (interuniversity cardiology institute) whereby patients receive enriched peripheral blood or bone marrow derived stem cells after a myocardial infarct. A new project on dendritic cells has been started in collaboration with Marieke van Ham (Sanquin Research, Dept of Immunopathology).

Key publications

Macaulay IC*, Tijssen MR*, Tijssen-Timmer DC, Gusnanto A, Steward M, Burns P, Langford CF, Ellis P, Dudbridge F, Zwaginga JJ, Watkins NA, van der Schoot CE, Ouwehand WH. Comparative gene expression profiling of *in vitro* differentiated megakaryocytes and erythroblasts identifies novel activatory and inhibitory platelet membrane proteins. *Blood* 2007; 109(8):3260-9.

*Both authors contributed equally.

Tijssen MR, van der Schoot CE, Voermans C, Zwaginga JJ. Clinical approaches involving thrombopoietin to shorten the period of thrombocytopenia after high-dose chemotherapy. *Transfus Med Rev* 2006; 20:283-93.

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Factors affecting proliferation and differentiation of stem and progenitor cells

This project aims to understand in more detail the expression, regulation and function of genes controlling proliferation and differentiation of megakaryocyte (MK) progenitor cells. During the *ex vivo* expansion process, CD34+ cells are stimulated with recombinant cytokines *in vitro* to generate partially differentiated megakaryocytic progenitor cells. This expanded population of more differentiated progenitors might be of use to reduce the period of thrombocytopenia after autologous stem cell transplantation. In our current work we have investigated the role of mTOR and the transcription factor STAT5 during MK progenitor expansion and differentiation.

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Role of mTORC1 and mTORC2 during megakaryopoiesis

Translational regulation plays a central role in cell proliferation, survival and cell differentiation through activation of the target of rapamycin (mTOR) signaling pathway. As the regulation of cell number and cell size are important factors during megakaryopoiesis, we investigated the role of mTOR signaling in Tpo-induced proliferation and differentiation using the specific mTOR inhibitor rapamycin. The downstream effectors of mTOR, p70 S6 kinase (p70 S6K) and 4E-binding protein (4E-BP) are phosphorylated by Tpo in a rapamycin- and LY294002-sensitive manner. Treatment of CD34+ cells and primary MKs with rapamycin inhibits Tpo-induced cell cycling and blocks cells in G₀/G₁. Furthermore, rapamycin markedly inhibits the clonogenic growth of MK progenitors with high proliferative capacity, but does not reduce the formation of small MK colonies. Addition of rapamycin to MK suspension cultures reduces the number of MK cells, but inhibition does not significantly affect expression of MK-specific glycoprotein's IIb/IIIa and Ib, nuclear polyploidization levels or cell survival. Summarizing, the results demonstrate that the rapamycin-sensitive mTOR pathway is activated by Tpo and plays a critical role in regulating proliferation of MK progenitors, without affecting differentiation or cell survival. Recently, it has been shown that the mTOR protein kinase is the key component of a more complex pathway and consists of two separate branches of the mTOR network: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1 contains mTOR in association with raptor and G L (or mLST8) proteins and

controls the phosphorylation status of proteins involved in initiating translational control, such as p70S6K and 4E-BP. mTORC2 contains mTOR in association with G L and rictor (or mAVO3) proteins and regulates PKB/Akt activation and the actin cytoskeleton. Until recently, work on mTOR has focussed mainly on the role of mTORC1 as the small molecule inhibitor rapamycin, in complex with the intracellular protein FKBP12, specifically inhibits the kinase activity of mTORC1. Although FKBP12-rapamycin does not bind to mTORC2, it does bind to free mTOR and prolonged treatment with rapamycin can inhibit new formation of mTORC2. In our current work we are investigating the roles of the individual components of the mTOR network during megakaryocyte proliferation and differentiation using small interference RNA targeting Raptor and Rictor.

Key publication

Drayer AL, Olthof SG, Vellenga E. Mammalian target of rapamycin is required for thrombopoietin-induced proliferation of megakaryocyte progenitors. *Stem Cells* 2006; 24:105-14.

Signal transducer and activator 5 (STAT5) is a negative regulator of megakaryopoiesis

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The transcription factor STAT5 plays a critical role in self-renewal and lineage commitment of hematopoietic stem cells (HSC's). It has been shown that persistent activation of STAT5 in CD34+ cells results in enhanced self-renewal and induction of erythroid differentiation, while myelopoiesis is severely impaired. In this study we analyzed the function of STAT5 during MK differentiation. Using RNA interference we downregulated STAT5 expression in peripheral blood CD34+ cells. Cells were transduced with a lentiviral construct encoding eGFP and a short-hairpin RNA for STAT5 or with a control vector. Transduction efficiencies were determined by flow cytometric detection of eGFP and ranged from 40-80%. Decreased STAT5 expression was verified by Western blot and quantitative RT-PCR (65% lower expression in STAT5 RNAi cells versus YFP+ control cells). To assess the effects of downregulation of STAT5 on the progenitor pool, the eGFP+ population was sorted and enumerated in colony assays. Downregulation of STAT5 significantly increased the number of MK progenitors (1.9-fold, $p=0.02$) and resulted in a decrease of erythroid progenitors

(0.6-fold, $p < 0.01$), but did not affect the number of granulocyte/monocyte progenitors. Prospective isolation of the common myeloid progenitor (CMP), the granulocyte/monocyte progenitor (GMP), the MK/erythrocyte progenitor (MEP), and the unilineage MK and erythrocyte progenitors demonstrated that downregulating STAT5 induced megakaryopoiesis in the CMP fraction, and downregulated erythropoiesis in the MEP unilineage erythroid progenitor fractions. To analyze the role of STAT5 during MK differentiation, transduced cells were cultured in serum-free medium containing Tpo and SCF and scored for cell counts and expression of MK markers weekly. The percentage of transduced cells and their number did not differ when STAT5 RNAi cells were compared to controls, indicating that downregulation of STAT5 provides no proliferative advantage or disadvantage during MK suspension culture. However, the expression of glycoproteins IIb/IIIa (CD41) and Ib (CD42) was upregulated, and an increase in large, polyploid cells was observed in STAT5 RNAi cells (15.2% polyploid cells versus 4.9% in control cells at day 7, $p < 0.01$). RT-PCR analysis of transcription factors predominantly expressed in MK and erythroid lineages revealed that Runx1 and Erg were increased in STAT5 RNAi cells, and the level of Tal1/Scl and p21 were decreased compared to control cells. These observations correlate with the increased MK differentiation observed in STAT5 downregulated cells. Together, these data demonstrate that the expression level of STAT5 has important effects regarding the type of hematopoietic cell development, with high levels favouring erythroid development and low levels enhancing MK differentiation and maturation.

The research described above is performed in collaboration with the Hematology Research Laboratory, University Medical Center Groningen (head Prof E Vellenga).

Cord blood

The purpose of this research is to increase the applications of cord blood (CB) for transplantation and transfusion. Experimental and clinical research concerns transfusion, *ex vivo* expansion and differentiation of CB stem cells (SC) and improvement engraftment. Research includes quality control, CB collection methods, product validation and storage.

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***In vitro* and animal experiments**

Research on CB transplantation focuses at manipulation of the CB SC in order to increase the number of adult patients that can be transplanted with CB SC. For this purpose three options (possibly combined) are promising: *ex vivo* expansion, co-transplantation with mesenchymal stem cells (MSC) and multiple donor transplantation.

With regard to *ex vivo* expansion of CB SC, we observed that Tpo as single growth/differentiation factor triggers proliferation of the SC but also differentiation towards megakaryocyte precursor cells. We showed that platelet recovery in NOD/SCID mice was significantly faster after transplantation with Tpo expanded cells compared to unmanipulated CB SC. In this line, we continued our research by studying the possibility to store *ex vivo* expanded cells by cryopreservation. This would simplify the logistics for transplantation and increase the safety of the transplant, including tests for microbial /bacterial contamination after expansion prior to transplantation. We found that that cryopreservation of Tpo expanded cells is possible, without negative influence on progenitor outgrowth.

In the NOD/SCID model we continued the evaluation of the effect of co-transplantation of MSC and Tpo expanded cells on speed of peripheral blood (platelet) hematopoietic recovery and the level of donor engraftment in the bone marrow. We observed no effect on the rate of platelet recovery by MSC. But, MSC resulted in better engraftment both for *ex vivo* expanded cells as well as for non-expanded SC. The exact nature of these kinetics are subject of ongoing investigation.

In addition, we examined the effect of *ex vivo* expansion of CB MNC with Tpo or Tpo in combination with a cytokine cocktail on the immunological composition of the graft. We observed that the cell composition of the expanded graft changes during expansion and that this is highly dependent on the cytokine cocktail used. Accordingly, this may have implications for the immunological activity of the graft and suggest that the choice of cytokines can be used as a tool to manipulate the composition of the graft in a primarily graft promoting or immunogenic direction.

Product development

After validating the manufacturing of CB collection with a special separation device (Sepax; Biosafe) we showed that it was possible to process and store a cord blood derived red blood cell (RBC) transfusion product for up to 21 days in SAGM with similar quality control parameters as standard products at the end of shelflife. However, as the contamination of leukocytes is higher than allowed according to the Dutch Guidelines for Blood Products. We evaluated filtration as a method of leukodepletion of the RBC fraction. This resulted in RBC loss over 40%, while storage results indicated that cell damage occurs during filtration, impairing storage time below 21 days in SAGM.

Future studies will further concentrate on improvement of CB red blood cell products, aiming at leukodepletion and increase of pH. The purpose is also to explore the use of allogeneic cord blood RBC transfusions.

Clinical research: The use of autologous cord blood for anemia of prematurity

This randomized clinical trial was started in 2004 and supported by The Dutch MRC. In this study premature neonates (gestational age 24-32 weeks) were allocated to a standard allogeneic RBC product or an autologous RBC product from CB. The study was performed at the Leiden UMC and simultaneously a pilot study was performed in the UMC Utrecht.

During the whole study period, 105 (LUMC) and 155 (UMCU) premature neonates, respectively from 229 and 255 evaluable neonates, received at least one RBC transfusion. The mean hospital stay of transfused prematures was in 29 ± 24.7 days (LUMC) and 33.9 ± 22.1 days in the UMCU. In an interim analysis after 21 months, the 68 randomized premature neonates in Leiden not showed a significant difference in median hospital stay between both arms. We stopped the study on October 1, 2006, because cost-effectiveness could not be determined. It was concluded that a significant reduction in allogeneic transfusions was not feasible due to low successful UCB collection rate and subsequently low product availability for the prematures that required the most transfusions. Interim calculations predicted that the study should be extended to 59 months to detect a difference. The results of the RCT can be submitted for publication in 2007.

Collaboration for the research projects described above are: WE Fibbe and E Goulmy (Dept of Immunohematology and Blood Transfusion, LUMC, Leiden); HHH Kanhai, S Scherjon (Dept Obstetrics, LUMC, Leiden); F Walther (Dept Pediatrics, LUMC, Leiden); GCML Christiaens (Dept Obstetrics, UMCU, Utrecht); HAA Brouwer (Dept Pediatrics, UMCU, Utrecht).

Key publications

Van Hensbergen Y, Schipper LF, Brand A, Slot MC, Welling M, Nauta AJ, Fibbe WE. *Ex vivo* culture of human CD34+ cord blood cells with thrombopoietin (Tpo) accelerates platelet engraftment in an NOD/SCID mouse model. *Exp Hemat* 2006; 34:943-50.

Moroff G, Eichler H, Brand A, Kekomaki R, Kurtz J, Letowska M, Pamphilon D, Read EJ, Porretti L, Lecchi L, Reems JA, Sacher R, Seetharaman S, Takahashi TA. Biomedical Excellence for Safer Transfusion (BEST) Collaborative. Multiple-laboratory comparison of *in vitro* assays utilized to characterize hematopoietic cells in cord blood. *Transfusion* 2006; 46(4):507-15.

Jansen M, Brand A, von Lindern JS, Scherjon S, Walther FJ. Potential use of autologous umbilical cord blood red blood cells for early transfusion needs of premature infants. *Transfusion* 2006; 46(6):1049-56.

Helming AM, Brand A, Wolterbeek R, Tol MJD, Egeler M, Ball LM. ABO incompatible stem cell transplantation in children does not influence outcome: a single center, retrospective analysis. *Pediatr Blood Cancer* 2006, Epub ahead of print.

Research on cellular blood products

Collection of autologous blood products by double erythrocytapheresis-cost effectiveness study

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The collection of autologous blood differs in some important aspects from homologous blood collection given the limited time window before an elective operation during which a number of 2-4 packed cell units can be collected without lowering the donor/patients preoperative hemoglobin. Currently full whole blood collection by phlebotomy is applied which limits the maximal number of units can be collected. An alternative approach is a double erythrocytapheresis, a procedure that can collect per donation session minimally 2 units of erythrocytes. This procedure also preserves the valuable blood components of the patient, such as plasma proteins, platelets, clotting factors and leukocytes and the patient receives compensation for the removed volume by saline or protein solutions, which makes this approach viable especially for older patients and patients with severe cardiac disease that would otherwise be unfit for a classical blood letting.

The project has determined the advantages of double erythrocytapheresis in the collection of pre-operative autologous erythrocytes as compared to the standard collections of whole blood and was performed in collaboration with Atrium Medical Center Heerlen, Maasland Hospital Sittard, Laurentius Hospital Roermond and Viecuri Medical Center North Limburg. Erythrocytapheresis was performed on the Haemonetics MCS+ equipment. In total 49 patients have been included for donation with double erythrocytapheresis and 52 patients have donated autologous blood by classical whole blood collection. From the 49 patients in the erythrocytapheresis group 3 were excluded because of: bad veins (1), fear of procedure (1), medical reasons (1). From the 52 patients in the whole blood group 4 were excluded because of: bad veins (2), fear of procedure (1), medical reasons (1). The percentage of successfully collected products by either method was comparable (90 versus 91%). In the erythrocytapheresis group 110 units were requested: 100 were collected in 50 erythrocytapheresis procedures. In the whole blood group 113 units were requested: 102 were collected in 102 whole blood procedures. The risk of donor/medical complications between erythrocytapheresis

and whole blood estimated by odds ratio was 1.21 (95% CI 0.70–2.10). All these complications fell in the category light (hematoma, dizziness and mild citrate reactions). The percentage of technical errors was higher in the erythrocytapheresis group, 4% (at the start of this project) versus 1% in the whole blood group. Though donors collected by erythrocytapheresis had significant lower pre-operation Hb (8.0 mmol/l) when compare to whole blood donors (8.8 mmol/l)($P < 0.001$), less autologous transfusions were used in this group. The costs to obtain a minimum of two units of autologous erythrocytes by double erythrocytapheresis are comparable to an equivalent number of units obtained by whole blood donations (double erythrocytapheresis < 5% more expensive). At this moment we can conclude that double erythrocytapheresis seems a feasible, well tolerated, safe and efficient procedure to collect autologous erythrocytes.

The Sanquin Blood Bank South-East Region has currently validated Erythrocyte products collect by double erythrocytapheresis. The purpose of the validation was to show that leukoreduced (by filtration) erythrocyte products collected by double erythrocytapheresis using the MCS+ van Haemonetics, meet the specifications for leukodepleted erythrocyte concentrates as described in the Dutch Guidelines for Blood Products. This study, performed in collaboration with Dr D de Korte (Dept of Blood Cell Research of Sanquin Research), showed that the processing of donor blood by Haemonetics MCS + to produce double RCC units from one donor, revealed no significant differences with respect to the composition of the red cell concentrate compared to RCC from whole blood donations. Levels of ATP after preparation and after 35 days of storage are significantly lower than standard RCC prepared from whole blood donations, whereas levels of 2, 3-DPG after preparation and in the first week of storage are clearly higher. This might be related to a slightly higher pH of double erythrocytapheresis RCC compared to standard RCC. However, based on the results for changes in the total adenine nucleotide pool analysis, it might be concluded that the double erythrocytapheresis RCC products meet the requirements set by the Dutch guidelines.

Therapeutic erythrocytapheresis as treatment for Hereditary Hemochromatosis patients

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Hereditary Hemochromatosis (HH) is a genetic disorder of iron metabolism resulting in excessive iron overload. Currently, phlebotomy is the standard effective treatment that prevents progression of tissue damage. Aim of the therapy is to reach ferritine levels between 20-50 µg/L. In patients with total iron stores of more than 30 grams, intensive treatment by means of up to weekly phlebotomies during 2 to 3 years is required to reach this aim. More recently mechanical removal of erythrocytes by therapeutic erythrocytapheresis (TE) has become a new therapeutic modality. By TE up to 1000 ml erythrocytes can be removed per session, depending on patient characteristics, compared to 300 ml erythrocytes per phlebotomy.

TE thus potentially offers a more efficient method to remove iron overload with less procedures in a shorter treatment period.

In this project, started in 2005, we plan evaluate the effectiveness of erythrocytapheresis against phlebotomy, both regarding the impact on the reduction in iron overload as well as the reduction in patient 'burden' in a randomized setting with a total number of 38 patients. Aspects of cost effectiveness will be included in the final analysis. The results of the study would allow decision-making based on Evidence Based Medicine on the 'best' therapeutic options available for newly-diagnosed as well as existing Hereditary Hemochromatosis patients.

A total of 25 patients eligible and willing to be randomized were included in the trial and treated yet. As a direct spin-off from the start of the project, the Dept of Internal Medicine of the University Hospital of Maastricht in collaboration with the Sanquin Research and Education Dept started a special out-patient clinic for hemochromatosis patients. Also, in collaboration with the Dept of Internal Medicine of the University Hospital of Maastricht, we have started a more extensive medical evaluation/follow-up as to the clinical effectiveness of the two treatment modalities, by more extensive cardiologic and/or hepathologic work-up by the participating medical specialists treating these patients. A Maastricht University hospital based study on incidence and prevalence of hemochromatosis in the Sanquin South East

Region will in due time allow to estimate the expected maximal yearly workload required from the blood bank to provide assistance in the therapy of iron overloaded patients.

Key publications

Rombout-Sestrienkova E, Loots W, van Deursen CThBM, Nillesen-Meertens AEL, Koek GH. Hereditaire hemochromatose. Ned Tijdschr Hematol, in press.

Rombout-Sestrienkova E, van Noord PAH, van Deursen CThBM, Sybesma BJPH, Koek GH. Therapeutic erythrocytapheresis versus phlebotomy in the initial treatment of Hereditary Hemochromatosis: A Pilot study. Transf Apheresis Sci. In press.

Reduction of blood transfusion in orthopedic surgery

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In orthopedic surgery several new approaches to reduce blood transfusions and to improve wound healing are available. In order to make evidence based choices on the usage of epoietin, several forms of autologous shed blood re-infusions, large multi-arm and multi-center studies are needed. The basic requirement for such studies is a strict transfusion protocol. We showed in a randomized controlled study in three orthopedic centers that a uniform transfusion trigger is feasible and can be applied among hospitals. In another study, the use of postoperative autologous shed blood re-infusion systems was feasible and safe. A multi-center study was designed on integrated blood sparing approaches (Optimal Blood Management (Transfusie Op Maat study – TOMaat –). Because of the complex design the study started as a feasibility study in 2004, supported by Sanquin. From May 2004 to December 2005 two participating hospitals (Leiden University Medical Center and Albert Schweitzer Hospital, Dordrecht) included 455 patients (inclusion percentage of 85%) showing that the design is feasible. In July 2005 a grant from The Dutch MRC was received as co-sponsoring to enable the TOMaat study for the entire 5 years (until January 2009). In 2006 two more hospitals participated: Slotervaart Hospital, Amsterdam (Feb 2006) and Groene Hart Ziekenhuis, Gouda (December 2006). These four hospitals resulted in a total of 1010 patient inclusions at the end of 2006. In December 2006 a progress report was approved by The Dutch MRC to continue study-sponsoring. In 2007 an interim analysis is planned.

Key publications

So-Osman C, Nelissen RGHH. De Transfusie Op Maat studie-optimaal bloedmanagement binnen de orthopaedische chirurgie. *Ned Tijdsch Orthop* 2006; 13:159-62.

So-Osman C, Nelissen RGHH, Eikenboom HCJ, Brand A. Efficacy, safety and user-friendliness of two devices for postoperative autologous shed red blood cell re-infusion in elective orthopaedic surgery patients. A randomized pilot-study. *Transf Med* 2006; 16(5):321-8.

Clinical effects of the peri-operative use of, by-filtration leukocyte reduced, red cell transfusions

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The peri-operative use of blood products has been researched in cardiac surgery setting, in vascular surgery, orthopedic surgery, and oncologic surgery.

In an international effort we are creating a combined database from RCT's investigating the effects of by-filtration leukocyte reduced transfusions in cardiac surgery patients. The authors of the RCTs (Brand, Bilgin, Boshkov, Wallis & van de Watering) and the independent statistician (EC Vamvakas), have compared and discussed the initially collected data elements, and agreed on the data elements that are to be entered in the newly combined database. We need, for one of our trials (van de Watering et al. *Circulation* 1998), to extend the follow-up period beyond the initially reported 60 days. In 2006, the collection of these follow-up data has started, with the aim to also report on the 10 year follow-up.

The collection of follow-up data for analysis of long term survival after gastrointestinal oncology surgery among the patients of the TacTicS-trial (van Hilten et al. *BMJ* 2004) was continued in 2006. For the vascular surgery patients of the TacTicS-trial the collection of follow-up data was started.

Key publication

Van de Watering L, Lorinser J, Versteegh M, Westendorp R, Brand A. Effects of storage time of red blood cell transfusions on the prognosis of coronary artery bypass graft patients. *Transfusion* 2006; 46(10):1712-8.

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Studies of the clinical efficacy of platelet products

The use of platelet concentrates (PC) for the prevention and treatment of bleeding complications in patients with thrombocytopenia, due to cytotoxic therapy or malignancies of the bone marrow, is generally accepted. Several new platelet products are under development. Although some of these products are used clinically, there is a lack of informative studies for a selection for one of these products. Moreover, observed differences in *in vitro* quality parameters correlate inconsistent with clinical efficacy. i) A recent study comparing platelets stored in an additive solution (PAS-2) with platelets stored in plasma showed comparable clinical efficacy. ii) A striking effect of several patient factors on clinical efficacy was noted, annihilating product related factors.

Two important issues are driving the current direction of PC development: prolongation of storage time, maintaining or even increasing safety in regard to contamination with pathogens. A clinical trial concerning these issues will be started in the beginning of 2007. iii) In this trial platelets stored in an additive solution (PAS-3) treated with or without a photochemical pathogen reduction procedure (PR-PAS-3), stored up to 7 days, will be compared to platelets stored in plasma. The results of this study will be used to design a quality monitoring protocol for the testing of PCs before transfusion.

Key publications

Kerkhoffs JL, Eikenboom JC, Schipperus MS, van Wordragen-Vlaswinkel RJ, Brand R, Harvey MS, de Vries RR, Barge R, van Rhenen DJ, Brand A. A multicenter randomized Study of the efficacy of transfusions with platelets stored in platelet additive solution II versus plasma. *Blood* 2006; 108:3210-5.

De Korte D, Curvers J, de Kort WL, Hoekstra T, van der Poel CL, Beckers EAM, Marcelis JH. Effects of skin disinfection method, deviation bag and bacterial screening on clinical safety of platelet concentrates in the Netherlands. *Transfusion* 2006; 46:476-85.

Schonewille H, Brand A. Prevention and diagnosis of delayed haemolytic transfusion reactions. *Vox Sanguinis International Forum* 2006; 91:361-2.

So-Osman C, Nelissen RGHH, Eikenboom HCJ, Brand A. Efficacy, safety and user-friendliness of two devices for postoperative autologous shed red blood cell re-infusion in elective orthopaedic surgery patients. A randomized pilot-study. *Transf Med* 2006; 16(5):321-8.

Clinical transfusion studies

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We continue our cooperative clinical research in induction and management of alloimmunization by blood transfusions and pregnancy, with emphasis on conditions in pregnancy. We identified that patients who developed red cell antibodies upon a first transfusion event are high responders towards a second event. This observation may be helpful to identify patients eligible for future extended matched RBC transfusions. This will be investigated in a randomized study. Another group for whom extended matching is urgently needed are females receiving intra uterine transfusions. Despite Rh-K-matching they develop broad RBC alloimmunization, similar as polytransfused patients with hemoglobinopathie.

With respect to alloimmune thrombocytopenia in pregnancy we continued our non-invasive approach, accumulating evidence that outcome compares favourably with more invasive approaches.

Key publications

Baumgarten R, van Gelder W, van Wintershoven J, Maaskant-van Wijk PA, Beckers EAM. Recurrent acute hemolytic transfusion reactions by antibodies against Do (a) antigens, not detected by crossmatching. *Transfusion* 2006; 46:244-9.

Van den Akker ES, Oepkes D, Brand A, Kanhai HH. Vaginal delivery for fetuses at risk of alloimmune thrombocytopenia. *Brit J Obstet Gyn* 2006; 113:781-3.

Kanhai HH, van den Akker ES, Walther FJ, Brand A. Intravenous immunoglobulins without initial and follow up cordocentesis in alloimmune fetal and neonatal thrombocytopenia (FNAIT) at high risk for intracranial haemorrhage. *Fetal Diag Ther* 2006; 21:55-60.

Brand A, Novotny V, Tomson TAS. Platelet Transfusion Therapy: from 1973 to 2005. *Hum Immunol* 2006; 67:413-8.

Te Pas AB, Lopriore E, van den Akker ES, Oepkes D, Kanhai HH, Brand A, Walther FJ. Postnatal management of fetal and neonatal alloimmune thrombocytopenia: the role of matched platelet transfusion and IVIG. *Eur J Pediatr* 2006. Epub ahead of print.

Schonewille H, van de Watering LM, Brand A. Additional red blood cell alloantibodies after blood transfusions in a nonhematologic alloimmunized patient cohort: is it time to take precautionary measures? *Transfusion* 2006; 46:630-5.

Schonewille H, van de Watering LM, Loomans DS, Brand A. Red blood cell alloantibodies after transfusion: factors influencing incidence and specificity. *Transfusion* 2006; 46(2):250-6.

Trannoy L, Terpstra FG, de Korte D, Lagerberg JW, Verhoeven AJ, Brand A, van Engelenburg, FAC. Differential sensitivities of pathogens in red cell concentrates to Tri-P(4)-photoinactivation. *Vox Sanguinis* 2006; 91:111-8.

Donor studies

The systematic recruitment of new blood donors

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The results of the studies onto the determinants of blood donation intentions showed that affective attitude, subjective norm, descriptive norm, self-efficacy, and personal norm are the most important predictors of blood donation. Based on these results we decided to focus on 2 strategies for improving donor recruitment. The first strategy is improving the Sanquin recruitment leaflet. A content analysis showed that these leaflets are more focused on raising knowledge instead of recruitment. Information targeting affective attitude, self-efficacy, and personal norm was barely included in the leaflet. A subsequent experiment among university students showed that the leaflet was not effective in motivating non-donor students to become blood donors. To improve recruitment, we want to add information targeting affective attitude, self-efficacy, and personal norm.

The second strategy to improve donor recruitment focuses on 'donors recruiting new donors'. A study onto the determinants of 'donors recruiting new donors' was conducted to see if donors were willing to participate in this and to study what factors influence this willingness. Donors received a questionnaire measuring this either at home or at the blood bank. The results showed that 53% of the donors at home had a positive intention to participate, compared to 59% of the donors at the blood bank. For donors at home, the most important predictors of the intention to participate were self-efficacy, cognitive attitude, and having positive experiences at the blood bank. For donors at the blood bank the most important predictor also was self-efficacy, followed by affective attitude, a personal norm to donate blood, and feeling responsible to help donor recruitment.

Based on these results we designed an information leaflet for donors asking them to participate in a 'donors recruiting new donors' campaign and targeting self-efficacy, affective attitude, and cognitive attitude (the three most important predictors). 'Donors recruiting new donors' postcards are designed to be distributed among potential donors (by participating blood donors) and can be filled out to register as a blood donor. Participants in this study will be assigned to 1 of 3 conditions, depending upon their arrival at one of the participating blood centers. At the registration desk they either receive an envelop with the 'donors recruiting new donors' infor-

mation leaflet and the postcards, or an envelop with the postcards only, or nothing (standard blood bank practice). All blood donors receive three questionnaires. The first questionnaire is send one week prior to donation, the second questionnaire one week after donation, and the third questionnaire 6 weeks after donation. The questionnaires measure the determinants of intention and intention to participate. The last questionnaire measures whether donors have recruited new donors among their family and friends (self-reported behaviour). This study will start 1 May and last until 8 June. The last questionnaires will be sent on 27 July 2007.

Publications

Lemmens KPH, Abraham C, Hoekstra T, Ruiters RAC, De Kort WLAM, Brug J, Schaalma HP. Why don't young people volunteer to give blood? An investigation of the correlates of donation intentions among young adults. *Transfusion* 2005; 45:945-55.

Development and evaluation of theoretically founded interventions aimed at donor retention

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A qualitative study which comprises in-depth interviews with donors and ex-donors has been completed. The interviews served to gain insight into motivations to start and stop donating blood. Differences in beliefs on donating blood between former and active donors were also explored. The results indicated an important influence of dealing with negative experiences (tense, fainting, deferral), difficulty in planning, missing a feeling of commitment and not feeling needed as a donor. The results are currently described in a scientific article.

Based on the insights gained from these interviews and the existing knowledge present in the theoretical debates on donor retention, questionnaires were constructed. We started with a questionnaire study into the willingness of voluntarily withdrawn donors to pick up donating again. This study should give us insight in important motivational factors for people to eventually continue donating or not. Questionnaires (N=800) were sent out in February 2007. The response rate is 43% and the results are currently being analyzed.

Donor Cohort Studies

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Donors from the essential starting point in the transfusion chain from donor to patient. Although much research has been done on blood and blood products, few studies have focussed their attention on the donor. Donor research is essential for establishing and maintaining a high qualitative blood supply. The main goal of this project is therefore to know more about our donors. The objectives are to gain insight into (1) the characteristics of the donor population and (2) into the efficiency of different processes in the blood bank that involve donors. With respect to donor characteristics, research will be aimed at investigating aspects such as the socio-economic background of donors, lifestyle, nutrition, medical history, donor motivation and physical activity. Concerning the efficiency of the blood bank donor processes, research questions will address the dynamics of the donor population. What are the processes that play a part in becoming a regular donor? How many donors do become regular donors and for what reasons? Why do people resign? What is the overall main deferral rate, and does it differ between various donor groups?

In order to gain insight into the above stated questions and research lines, a dynamic cohort containing approximately 10% of the national blood donor population will be formed. In this cohort study, whole blood and plasma donors will be followed in time using both questionnaires and routinely gathered blood bank data. Much of our research activities in 2006 were aimed at constructing and organizing the cohort study. Permission from the Central Committee on Research Involving Human Subjects (CCMO) for the execution of the study was obtained. The initial questionnaire was extensively modified, based on the results of the first pilot study. In addition, invitation letters and information brochures were developed. Furthermore, a solid logistic framework was built, necessary for successful start and continuation of the cohort study. Research assistants were recruited and instructed for assistance in the cohort study. In October 2006 the logistics of the cohort study were completed. Due to the extensive changes of the questionnaire, it was decided to do a second pilot study among 250 donors. This mailing yielded a response rate of 69%. Eight percent of the donors indicated that they were not willing to

participate in the cohort study and twenty-three percent did not respond. Ninety-eight percent of the included donors indicated that they are willing to participate in future research. Currently, the data collection for the large cohort Donor InSight has started and questionnaires will be sent out regularly.

Scientific Contributions to the following projects involving donor management

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Retention of new donors

The results of a conducted qualitative study under former donors gave input to the programme 'Retention of new donors'. The programme has been developed by a project group directed by the Manager Donor Affairs in the South East Region. This new programme is currently being evaluated at two donor centres in the South East and South West Region of the Netherlands. The programme will be evaluated by means of donor retention statistics and by questionnaires measuring (a) differences in donor satisfaction and (b) psycho-social determinants of donation behaviour, between test and control centres.

Passage times

In 2006, a study on passage times of the donation process has been conducted in all blood banks in the South East Region in The Netherlands. The study leads to an overview of passage and waiting times during the donation process in blood banks. Results show that 65% of all whole blood donors pass through the donation process within 45 minutes. Beforehand, a percentage of 85% was pursued. Therefore, in 2007 intervention strategies will be developed in order to improve waiting and process times.

Reasons for voluntary withdrawal

We participated in a project group investigating reasons for withdrawal among voluntarily lapsed donors. Staff members of the callcenters in three regions classified withdrawal reasons during several weeks. The classification procedure resulted in an overview of withdrawal reasons, from which fifteen withdrawal reasons were chosen. These fifteen reasons can replace the two withdrawal-codes for voluntary withdrawal that are currently being used in Progesa.

Prediction of future Hemoglobin levels

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The aim of the proposed study is to predict future haemoglobin levels using a multivariable approach. Correct prediction of future Hb levels in whole blood and plasma donors will diminish the number of Hb deferrals. It will also help in maintaining a healthy donor population with respect to its iron stores and Hb levels, which is important in preserving (new) regular donors. The present study focuses on the following research objectives:

- (1) To assess and construct a prognostic model for future haemoglobin levels in whole blood and plasma donors. In particular, the study will investigate to what extent donor and donation information or variables already obtained in the blood bank information system provide prognostic knowledge for Hb level outcomes.
- (2) To investigate the added value of erythrocyte-zinc protoporphyrin (ZPP) measurements in the prognostic model.
- (3) To derive a simple prediction rule that will be applicable and easy to use in everyday blood bank practice.

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Research lines

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As an academic research group, publication of articles in scientific medical journals is the main objective, but internal reporting within Sanquin to allow informed decisions on strategic or policy issues is an additional task. The latter reports may not always be public. Finally, reporting in the European context is aimed at establishing and disseminating common standards and methodologies.

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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.

Project leader: H ter Hart (h.terhart@sanquin.nl)

For the manufacturing of Nanogam[®], Sanquin's liquid immunoglobulin product, Cohn fractionation paste II is used as starting material. Suitability studies for the use of paste II from third parties were finalized and it was shown that this paste II can be used for the manufacturing of Nanogam[®].

In close collaboration with Sanquin Research, a project to characterize liquid immunoglobulin products is ongoing. Studies on the nature of fragment formation were finalized and studies on dimer formation and polymerization are ongoing. Development work on a manufacturing process for transferrin was started.

Project leader: M Kleijn (m.kleijn@sanquin.nl)

Feasibility to implement a 15 nm Planova-filtration step in the manufacturing process of Cetor[®] to enhance the virus safety of this high purity C1-inhibitor product was shown and three large scale batches with this improved process were manufactured and characterized. Feasibility of the use of US plasma was shown. Stability studies are ongoing. Robustness studies on the virus reducing capacity of this new step were performed and showed excellent results. In close collaboration with Sanquin Virus Safety Services prion removal studies are started. A characterization program was developed to study impurities profiles of the intermediate products. Clinical studies in The Netherlands and USA with this virus safe C1-esteraseremmer-N were started in 2005 and will be finalized in 2007. In close collaboration with Sanquin Research, a project to characterize C1-inhibitor products was started.

Project leader: I Prins (i.prins@sanquin.nl)

Development work on a manufacturing process for a third generation liquid IVlg product was finalized. A process to manufacture a liquid IgG product comparable to Nanogam but with a higher process yield was developed.

Development work on a manufacturing process for transferrin was started.

Project leader: A Koenderman (a.koenderman@sanquin.nl)

Another project concerns a new potential anti-HIV agent, so called negatively charged albumin. The three clinical grade batches produced so far have shown excellent stability on storage. A proof-of-principal study in a limited number of AIDS patients was performed. Due to possible side-effects no further clinical studies will be done. This development project was executed in co-operation with the University Center for Pharmacy, University of Groningen and the International Antiviral Therapy Evaluation Center (IATEC) of the Academic Medical Center of Amsterdam.

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The strategy of the CAF-DCF Product Support Division (former R&D) focuses on both the efficacy of plasma-derived medicinal products and their safety as regards pathogens and environmental pollutants. The paradigm of blood safety is approached by a combination of donor selection, screening tests, the evaluation of critical epidemiological and pathogen-related data, and pathogen reduction.

Focusing on therapeutic proteins and their excipients in plasma or concentrates, the Division develops both immunological methods (e.g. FVIII epitope identification, detection of neutralising anti-B19 and neutralising total and strain-specific anti-pneumococcal antibodies in IVIG) and biochemical techniques (such as intrinsic and extrinsic protein fluorescence) and exploits them in industrial applications. Besides studies on UVC irradiation as a new virus inactivation technology developed by DCF-CAF R&D, we have run ex vivo experiments on a new potential immunomodulation application in association with a treatment using intravenous immunoglobulins at the patient's bedside. Research is still ongoing on Parvovirus B19. The aim is to distinguish in donors its infectivity (multiplication) from its detection by nucleic acid amplification techniques (NAT).

The Product Support Division is also dedicated to providing expert scientific and technical advice and to offering state-of-the-art analytical methods meeting the latest regulatory requirements.

Polyvalent Intravenous Immunoglobulins (IVIG), FVIII inhibitors, and immunomodulation with UVC

In silico prediction of FVIII epitopes recognized by natural autoantibodies in IVIG
Inhibitory antibodies directed against blood coagulation factor VIII (FVIII) impair FVIII replacement therapy, constituting a serious complication in haemophilic and autoimmune patients. Identifying B-cell FVIII epitopes and mapping them on the molecule remain important challenges. Using a combination of different algorithms, more than 30 hypothetical linear epitopes were predicted on the FVIII molecule surface. We selected several major predicted sequences, spanning all FVIII domains, for specific antibody induction in rabbits. All peptides tested successfully

induced production of specific anti-FVIII rabbit antibodies, supporting the relevance of our approach. To investigate the presence of FVIII-reactive antibodies in the healthy donor population, a pooled fraction rich in all IgG subclasses was purified on peptide-Sepharose columns. Substantial amounts of Ig, specific for each FVIII peptide, were purified with yields ranging from 8 to 223 ng/mg immunoglobulins. Our results confirm the diversity of FVIII epitopes recognized by natural human anti-FVIII autoantibodies. All IgG subclasses were found in the affinity-isolated anti-peptide material, with overrepresentation of IgG2 and IgG4. Evidence was also found for new FVIII epitopes. Five human anti-peptide preparations displayed FVIII-neutralising activity, ranging from 1.3 to 5.3 BU/mg. Although the presence of naturally occurring anti-FVIII antibodies in healthy donors has been previously described, our methodology has allowed, for the first time, fine mapping of several inhibitory and non-inhibitory epitopes. Our observations support the hypothesis that FVIII inhibitors in haemophilia A and autoimmune disease may originate from the proliferation of natural FVIII-specific B-cell clones.

Total pneumococcal antibodies in different IVIG preparations and as a tool to monitor IVIG infusion

Streptococcus pneumoniae is a leading cause of morbidity and mortality in both developed and undeveloped countries. Antibodies against capsular polysaccharides protect against disease, probably by inducing complement-mediated opsonophagocytic activity. Controlled studies indicate that the prophylactic use of IVIG is of benefit in patients with hypogammaglobulinaemia. A simple, specific, and reproducible ELISA has been developed to quantify specific antibodies against 23 strain-specific capsular polysaccharides in the starting plasma pools and the IVIG (Elizen, Zentech, Liège). The test was performed according to WHO guidelines after absorption of polysaccharide C or/and polysaccharide 22F. It was used to determine anti-pneumococcal antibodies in different preparations of IVIG (Nanogam, Multigam, Octagam, Sandoglobulin, Gammagard, and Intratect). It was successfully used to monitor IVIG infusion in bone marrow transplant children treated with IVIG. A protocol is designed to assess the protection of Multigam (CAF-DCF) against pneumococcal infection in immunodeficient paediatric patients. The study will be performed in collaboration with most Belgian paediatric centres.

Improved efficacy of UV-irradiated lymphocytes by intravenous immunoglobulins

IL-1Ra prevents IL-1-induced inflammatory signalling, a mechanism potentially important for several pathological conditions characterized by inflammation. When administered as a drug in the recombinant form, IL-1Ra displays a protective effect towards these conditions, so we have postulated that this action might also be achieved by pharmacological activation of endogenous IL-1Ra production. We have previously shown that photochemotherapy and UV light increase IL-1Ra secretion by monocytes/macrophages. A similar effect has been shown for IVIG. The aim of this study was to define optimal *in vitro* conditions for induction of IL-1Ra secretion. As both agents induce lymphocyte apoptosis, we focused our analysis on the influence of IVIG- and UV-induced IL-1Ra production on this mechanism. After overnight pre-incubation at 37°C, UV-irradiated PBL mixed with IVIG at two concentrations (1 and 25 mg/ml) were cocultured with autologous PBMC. Apoptosis was measured by detection of annexin V/PI. IL-1Ra secretion was evaluated by RT-PCR and a Luminex microbead array assay. A significant additive dose-dependent influence of IVIG (+85%; $p=0.0005$) on UV-induced IL-1Ra secretion was found to involve both Fc-receptor activation at low dosage (1 mg/ml) and a potent apoptotic action on PBL, reinforcing the UV effect at high concentration (25 mg/ml). We conclude that lymphocyte apoptosis is an important pathway contributing to IVIG-mediated enhancement of UV-induced IL-1Ra production by monocytes/macrophages and that these findings should be considered when evaluating *in vivo* protocols for photochemotherapy and IVIG treatment, in the hope of improving efficacy.

Evaluation of epidemiological data for HIV, HCV, and HBV

Surveillance of infectious markers in donor populations is important in order to recognize trends in the prevalence and incidence of transfusion-related infections. We have collected yearly the epidemiological data from all blood banks. According to EMEA/CPMP/BWP/125/04 guidelines, data pertaining to the last year and the three previous years were analyzed. We separately calculated transmissible-disease-positive rates for the 3 viral markers to identify any overall trend in the rates of infectious markers in first-time donors (FD) and repeat donors (RD). To assess the residual risk of the presence of agents for which donations were screened, we

applied what is known as the incidence rate – window period model. The analyses were performed for successive overlapping 2-year intervals from 2002 to 2005). The incidence rate was derived by calculating known confirmed incident cases detected by serological and NAT screening divided by the total number of RD donations and the mean inter-donation interval length. A total of 191,116 first-time donors for a total of 4 years were included in this study. First-time donations represent about 6% of the total donations in the plasma pool for fractionation. Prevalence levels for all three markers show a slight decrease from 2002 to 2005.

Incidence rates of HIV, HCV, and HBV and residual risks were also determined on a total of 968,385 repeat donors who donated 2,634,810 donations (mean 2.72 donations per donor and per year). The incidence rate was found to be constant for HCV over the three periods, with a very low residual risk (between 0.39 and 0.44 per million donations). The incidence rate for HIV was very low (maximum 0.396 per million donations) for all three periods. The incidence rate for HBV decreased significantly in the last period 2004-2005, with a risk of 5.93 per million. When HIV and HCV NAT tests were applied to mini-pools of eight donations in blood banks, the tests revealed 2 HCV-positive donations from first-time donors and 2 HIV-positive donations from repeat donors in 2005, showing an increase in viral safety at the level of donations.

B19 infectivity in hepatocarcinoma cells

Infectivity of B19 genotypes 1 and 2

Erythrovirus (formerly Parvovirus) B19, a very resistant human pathogen, is found in Belgian donors with a prevalence of 1/10,000 (mostly genotype 1). A strategy for reducing potential B19 transmission by plasma derivatives is based on NAT screening of mini-pools of 576 donations, identifying the positive donations (more than 5000 IU B19/ml), and discarding them. Measuring B19 infectivity in general, and particularly the residual infectivity present after screening for parvoviruses, parvovirus neutralization by plasma immunoglobulins, or virus inactivation, remains a major health challenge. B19 of both genotype 1 and genotype 2 was shown to multiply actively in hepatocarcinoma cells.

Longitudinal investigation of 19 B19-positive donors for specific anti-B19 antibodies and B19 infectivity

Seventeen donors (12 males and 5 females) with an initial B19 level higher than 10^5 IU/ml were monitored for 1 year. Two were first-time donors and 15 were repeat donors. Every donor was interviewed for clinical symptoms. The study started in 2006. Samples were collected and screened for B19 DNA and specific IgM and IgG antibodies. No good correlation was found between clinical symptoms and viral load. In about one-half of the donors, the B19 DNA load decreased rapidly by more than 5 logs during the first 12 weeks and then stabilized at a low background level for 1 year. In the other donors (where the initial B19 DNA load was lower), a slow 2-3-log reduction was measured. All donors actively developed anti-VP antibodies (IgG and even IgM) specific to linear and conformational capsid epitopes. Despite the presence of abundant anti-B19 IgG antibodies, persistent B19 infectivity was demonstrated in several cases, showing that live B19 virus persisting for more than one year is not a rare event.

Collaboration with P Caillet-Fauquet¹, L Craciun², Y de Launoit^{1,4}, E Dupont², ML Draps¹, M Goldman², KM Hourfar³, WK Roth³, M Schmidt³, E Seifried³, A Themann³.

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Caillet-Fauquet P, Di Giambattista M, de Launoit Y, Laub R. Does virus quantification provide a real measure of parvovirus B19 infectivity? *Journal of Clinical Virology* 2006; 37:77-8.

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The Medical Department is responsible for the design and conduct of clinical trials with (recently developed) plasma products in order to obtain marketing authorization or new indication(s). Therefore the Medical Department closely cooperates with clinical investigators in the Netherlands and abroad, e.g. the Dutch Inter-University Working Party on the Study of Immune Deficiencies and the Hemophilia Treatment Centers.

To ensure safety of the products the Medical Department is obliged to implement an appropriate system of pharmacovigilance to collect, collate and evaluate information about suspected adverse reactions of medicinal products. Pharmacovigilance is performed both passively based on received reports on adverse events and actively by performing post authorization safety studies (PASS) in ad random patient groups. Periodic Safety Update Reports (PSUR's) provide the authorities pharmacovigilance data. PSUR's have been prepared for Cealb[®]/Albumine 5%/G.P.O. and Aafact[®], TetaQuin[®], Immunoglobuline IV, VariQuin[®], HepBQuin[®], and Cetor[®] for a review period of 5 years, as part of re-registration requirements. An addendum to the PSUR for Cofact[®] was prepared, in order to obtain Marketing Authorization via a Mutual Recognition Procedure. Authorization for Cofact[®] was granted in Finland, Belgium, France, Luxembourg, Germany, Austria and Italy. The PSUR for Nonafact[®] was compiled for the fourth-and-a-half year review period.

Medical information and advice is provided to medical specialists, physicians, nurses and pharmacists on the optimal use of plasma products. Furthermore, the Medical Department assists in the recruitment of new plasmapheresis donors and performs for the Sanquin Blood Banks the selection of specific units of erythrocytes for immunization in order to obtain specific source plasma for the fractionation of Anti-Rhesus (D) Immunoglobulin.

Clinical trials ongoing in 2006

Nonafact®

The safety of the usage of Nonafact® in regular patient treatment is being assessed in a multi-center clinical trial, entitled 'Post marketing study in hemophilia B patients using Nonafact® 100 IU/ml powder and solvent for solution for injection (human coagulation factor IX) (human plasma derived factor IX product, freeze dried)', in five Hemophilia Treatment Centers in the Netherlands. At the beginning of 2007 the observation period will be completed and the study report will be finalized in 2007.

Nanogam®

A multi-center, controlled, cross-over clinical study, 'Treatment in patients with recurrent infections and IgG Subclass Deficiency, and/or Deficient Anti-polysaccharide Antibody Response', designed to compare the efficacy of Nanogam® with the efficacy of antibiotics in the treatment of recurrent (upper respiratory tract) infections in patients with IgG-subclass deficiency or a deficient anti-polysaccharide antibody response was prepared in cooperation with the Dutch Inter-University Working Party on the Study of Immune Deficiencies. The start of the study is expected in seven centers in 2007.

In addition, a multi-center, randomized clinical study in patients with (chronic inflammatory demyelinating polyradiculoneuropathy, CIDP) is in preparation in order to investigate the efficacy of Nanogam® in newly diagnosed patients with Chronic Inflammatory Demyelinating Polyradiculoneuropathy (CIDP) and in patients with a relapse.

An investigator-initiated study, 'Desensitization of highly pre-sensitized dialysis patients waiting for kidney transplantation by Rituximab, Ivlg-L and rescue Plasmapheresis', will be conducted in order to assess the efficacy of Nanogam® treatment to reduce allo-antibody levels in patients awaiting renal transplantation in Dutch renal transplant centers.

PPSB-SD®

The efficacy of treatment with PPSB Solvent Detergent® was compared to the standard treatment with Fresh Frozen Plasma (FFP) in patients using oral anticoagulant therapy and undergoing acute cardiac surgery with a cardiopulmonary by-pass. The study was conducted and completed in the Academic Hospital of the Catholic University 'Gasthuisberg', Leuven in Belgium in collaboration with the Medical Department of CAF-DCF cvba. The results demonstrated that treatment with PPSB-SD reverses anticoagulation safely, more rapidly and effectively than FFP. A clinical study report was finalized in 2006 and two papers were presented at intensive care Conferences.

MBL

A clinical trial with Mannan Binding Lectin, (MBL, a product from Statens Serum Institut (SSI), Copenhagen, Denmark), entitled 'Phase II study on Mannan Binding Lectin (MBL) substitution in MBL-deficient children with chemotherapy-induced neutropenia', was performed. The objective was to investigate the pharmacokinetics and the clinical and biological effects of MBL replacement therapy in MBL-deficient children during chemotherapy-induced neutropenia. In the Academic Medical Center in Amsterdam 12 patients were included who received a total of 20 treatments. In December 2006 the study was completed. The clinical study report is in preparation.

C1-esterase inhibitor

To optimize viral safety, a 15 nm filtration was introduced in the production process of Cetor®, a highly purified C1-esterase inhibitor concentrate. This improvement of the manufacturing will be submitted as a variation for marketing authorization. Therefore, a multi-center study 'Pharmacokinetics, clinical efficacy and safety of C1 inhibitor concentrate (C1-esteraseremmer-N) for the treatment of hereditary (and acquired) angioedema' was set up in collaboration with the Academic Medical Center Amsterdam, Erasmus Medical Center Rotterdam, University Medical Center Groningen, University Medical Center St Radboud Nijmegen, and Haga Hospital The Hague. The study comprises three parts, pharmacokinetics (part A, phase II),

treatment of attacks of angioedema (part B, phase III) and prophylactic use of C1 inhibitor (part C, phase III). In part B + C, data on the efficacy and safety of C1-esteraseremmer-N will be collected. Part A of the study has been completed and the study report is in preparation. The results of this part provide evidence that the pharmacokinetic properties of C1-esterase inhibitor and Ceteror® are similar. Currently, part B and C of the study are ongoing and expected to end mid 2007.

Key publications

Strengers, PFW. Aanvallen van angio-oedeem bij kinderen door C1-esteraseremmerdeficiëntie. Ned Tijdschr Geneeskunde 2006; 150:1480-1.

Lavenne-Pardonge E, Amuli Itegwa M, Kalaai M, Klinkenberg G, Loncke JL, Pelgrims K, Strengers PFW. Emergency reversal of oral anticoagulation through PPSB-SD: the fastest procedure in Belgium. Acta Anaesth Belg 2006; 57:121-5.

Van Aart L, Eijkhout HW, Kamphuis JS, Dam M, Eeftinck Schattenkerk M, Schouten TJ, Ploeger B, Strengers PFW. Individualised dosing regimen for prothrombin complex concentrate more effective than standard treatment in the reversal of oral anticoagulant therapy: an open, prospective randomized controlled trial. Thrombosis Research, 2006; 118:313-20.

Demeyere R, Arnout J, Strengers P. Prothrombin complex concentrate versus Fresh Frozen Plasma in patients on oral anticoagulant therapy undergoing cardiac surgery: a randomized study. 26th Int. Symp. on Intens. Care and Emergency Med. (ISICEM), Brussel March 21-24, 2006. Critical Care 2006, 10 (Suppl 1):P233: S97-S98) (abstract).

Gillardin S, Demeyere R, Arnout J, Marcar J, Strengers P. Prothrombin Complex Concentrate versus Fresh Frozen Plasma in patients on oral anticoagulant therapy undergoing cardiac surgery with cardio-pulmonary bypass. A randomized clinical trial. 21th Europ. Ass of Cardiothoracic Anaesthes. Meeting (EACTA), Venice-Mestre. May 24-27, 2006 (abstract).

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Sanquin Reagents is among the first manufacturers of blood group and immune reagents in the world. By virtue of its research facilities and diagnostic laboratories, Sanquin developed a broad range of blood group and immune reagents, including several innovative products for diagnostic use and for fundamental and clinical research. Sanquin reagents are available worldwide through a network of distributors. Sanquin Reagents is ISO 9001 and ISO 13485 certified. Sanquin Reagents is committed to introduce new products on a continuous basis. New products are the outcome of R&D projects, some of which are executed in close collaboration with departments of Sanquin Research.

R&D projects

The project portfolio in 2006 consisted of ongoing projects in the fields of blood grouping and immunology reagents.

The following development projects were continued:

- (I) latex-based assays to quantify human IgG subclass species in blood for the Immage (Beckman Coulter).
- (II) assays to detect free, human immunoglobulin light chains (kappa, lambda) in blood.
- (III) a blood donor bloodgrouping chip based on DNA genotyping of red cell antigens.
- (IV) peptide MHC multimers (class I) with UV-cleavable peptides.
- (V) Pelicontrol.
- (VI) an assay to detect and quantify anti-myelin antibodies in blood of MS (Multiple Sclerosis) patients.
- (VII) an ELISA for quantifying human perforin in plasma.
- (VII) MASPAT: beads in stead of cells.
- (VIII) an instrument to automate the filling, closing, labeling and packaging of Cellbind cards.
- (IX) a dedicated line of antisera for the Magister.

Products and patents

New products

The following new products were commercially introduced in 2006:

- (I) Magister, a fully automated instrument for Cellbind gelcard testing was launched in Italy.
- (II) von Willebrand Factor propeptide ELISA reagents.
- (III) various white label reagents (as spin-off from Sanquin Research).

Patents

Ongoing patent applications: 1) cleavable peptides in MHC class I molecules and 2) multiplex PCR & specific DNA sequences that are used in the bloodgrouping chip.

Quality system

Two ISO certificates were renewed in 2006 (ISO 9001 & ISO 13485)

Key publications

Toebes M, Coccoris M, Bins A, Rodenko B, Gomez R, Nieuwkoop NJ, van de Kastele W, Rimmelzwaan GF, Haanen JB, Ovaa H, Schumacher TN. Design and use of conditional MHC class I ligands. *Nature Med* 2006; 12(2):246–51.

Rodenko B, Toebes M, Hadrup SR, van Esch WJ, Molenaar AM, Schumacher TN, Ovaa H. Generation of peptide-MHC class I peptides through UV-mediated peptide exchange. *Nature Protocols* 2006; 1(3):1120–32.

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Sanquin Pharmaceutical Services (SPS) is a business unit specialized in a broad array of pharmaceutical services aiming at the development 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.

Contract production

SPS has ample experience in designing production strategies and scaling up of production in compliance with EU and FDA guidelines. For this purpose SPS holds a GMP-license for the production of clinical grade pharmaceuticals including large scale fermentation, purification and sterile filling. The use of a specially developed serum-free culture medium in fermentation, guarantees a process free of concerns related to the transmission of pathogens such as prions and mammalian viruses. Several generic purification schemes for different types of proteins are available, allowing SPS to provide their clients already in an early stage of development with a validated process. In their multipurpose plant several projects can be handled simultaneously, allowing for fast turn around times.

Biosafety testing

SPS is also experienced in conducting a broad array of biosafety tests required for the pharmaceutical release of biotech products in compliance with both EU- and FDA guidelines. For this purpose, all assays have been GCLP (Good Control Laboratory Practice) accredited. Other QC services such as protein characterization, stability test programs, formulation studies and process validation (for demonstrating the reduction of (model) viruses or DNA during purification) as well as the validation of client dedicated assays are part of their dedicated activities.

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Sanquin Diagnostic Services offers routine and 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 for all Dutch Health Care Institutions and commercial companies. The division aims to work according to the highest quality standards in order to function as a diagnostic reference center in the fields mentioned above, in national as well as in 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 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.

Developments in 2006

A number of new tests and services has been introduced in 2006:

- Determination of the JAK2 mutation in patients with a myeloproliferative syndrome;
- A one-day MAIEA (monoclonal-antibody immobilization of erythrocyte antigens) technique for the detection of HTLA antibodies;
- A one-day MAIPA (Monoclonal Antibody-specific Immobilization of Platelet Antigens) assay for use in platelet alloantibody specificity determination;
- A functional assay for detection of heparin-dependent antibodies, the HIPAA (Heparin Induced Platelet Aggregation Assay);
- Assays for measuring the genotype, protein concentration and functional capacity of complement factor C4;
- A new quantitative C2- ELISA;
- Multiplex Ligation-dependent Probe Amplification (MPLA) technology for the diagnostics of rare forms of alpha and beta thalassemia;
- Light cyler technology for diagnostics in hemochromatosis;
- Band 3 protein by means of the flow cytometric EMA (eosin-5-maleimide) labeling test and a polyacrylamide gel electrophoresis (PAGE) separation of membrane proteins;

- A functional assay and an antigen assay of protein C;
- The screening system for Parvovirus B19 was improved so as to recognize the two new molecular variants, genotype 2 and 3.

In close collaboration with all involved departments, a specialized committee of Sanquin Diagnostic Services, dedicated to innovation of diagnostic services (called DC-I), has been active in 2006 in the following fields:

- Use of array technologies for diagnostic purposes;
- Complement research, focused on C1-esterase inhibitor, mannose binding lectin (MBL) and C4 pheno- and genotyping;
- Development of a single nucleotide polymorphism (SNP) platform.

In the following paragraphs, the above-mentioned developments will be described in more detail, ordered according to department.

Immunocytology

In 97% of Polycythemia Vera patients and in 20-50% of other myeloproliferative syndromes a JAK2 mutation occurs. In 2006, Sanquin Diagnostic Services introduced routine diagnostic testing for this mutation.

Supported by a grant from the KWF (Dutch Cancer Society) a new project has been started aimed at detection of minimal residual disease (MRD) in patients with neuroblastoma.

For the cytogenetic classification of B-CLL patients, a test based on Multiplex Ligation-dependent Probe Amplification (MPLA) will be developed and validated. A start has been made to integrate molecular diagnostics with other Sanquin departments, wherever possible.

Immunohematology

The focus of the department of immunohematology is to function as an (inter)national reference laboratory for erythrocyte, platelet and granulocyte serology, encompassing:

- identification of alloantibodies in transfusion and pregnancy;
- serologic examination in alloimmune cytopenia;

- serologic studies in TRALI and other immunological transfusion reactions;
- laboratory monitoring of pregnancies at risk for hematological disease of the foetus and newborn or neonatal alloimmune thrombocytopenia;
- screening of Rhesus D negative pregnant women on the presence of antibodies to erythrocytes during week 30 of the pregnancy.

During 2006, serologic assays have been developed in several of the above mentioned fields of interest:

- a one-day MAIEA (Monoclonal Antibody Immobilization of Erythrocyte Antigens) assay has been introduced for use in erythrocyte alloantibody specificity determination, especially for the detection of HTLA antibodies;
- a one-day MAIPA (Monoclonal Antibody-specific Immobilization of Platelet Antigens) assay has been introduced for use in platelet alloantibody specificity determination;
- a functional assay has been developed for detection of heparin dependent antibodies, the HIPAA (Heparin Induced Platelet Aggregation Assay);

Also in 2006, the Kleihauer test has been evaluated with the use of flow cytometry. From logistic, efficiency and cost considerations, it was concluded not to implement this test in the Dept of Immunohematology. Current efforts are dedicated to the validation and implementation of fetal genotyping in maternal plasma of K, Rhc, RhE and HPA-1a. For patient care in case of transfusion, genotyping of other blood group systems (amongst which the Knops system and high frequent antigens) will also be developed.

Autoimmune Diseases

For the detection of autoantibodies in diabetes, the anti-GAD65 and anti-IA-2 assays are routinely used in the Dept of Autoimmune Diseases. In 2006, accreditation by the Council of Accreditation was obtained for the anti-GAD assay; the anti-IA-2 test will hopefully be accredited in spring 2007.

For detection of antibodies to Extractable Nuclear Antigens (ENA), the in house immunoblotting test has been replaced with a commercial line blot that enables the

simultaneous detection of several autoantibodies in one test. To improve myositis diagnostics, a myositis blot has been implemented that allows the detection of several myositis-specific autoantibodies. A new autoantibody specificity for the diagnosis of autoimmune hepatitis has been added to our package (soluble liver antigen, SLA/LP).

For the detection of autoantibodies to aquaporine-4 in Neuromyelitis optica, an assay has been developed. Yet, in spite of recent publications, the assay has up till now not proven to discriminate patients well enough from controls.

In close co-operation with the Depts of Blood Cell Research and Immunopathology assays have been developed to measure the genotype (MLPA assay), protein concentration (antigen ELISA) and functional capacity of C4 (functional ELISA). Results of this study will be published. After validation, these assays will be introduced in routine diagnostics.

Immunochemistry

During 2006, a number of assays amongst which the CH50 and AP50 assays as well as the C4-deposition test have been automated. Measurement of serum levels of monoclonal therapeutics (Infliximab[®], Adalimumab[®] and Ethanercept[®]) has been implemented on a Summit ELISA robot.

Automated measurement of C1-esterase inhibitor on a TECAN 'Freedom Evo' ELISA processor is currently in pilot phase.

Measurement of IgG levels in blood samples obtained by an easy finger prick has proven successful, and has now been validated. A new quantitative C2- ELISA has been automated on the Genesis TECAN robot and will be validated early 2007.

In close collaboration with the Dept Product Development of Sanquin Plasma Products, a validation report has been written about the applicability of measuring five plasma proteins (IgM, albumine, ceruloplasmin, C3 and C5) in intermediary products during production of C1-esterase inhibitor (Cetor) from human plasma. Phenotyping of alpha-1-antitrypsin on the SEBIA robot has been validated. This new technique is more simple to use than the existing Amersham immobilin technique.

Blood Cell Chemistry

During 2006, the Multiplex Ligation-dependent Probe Amplification (MPLA) technique has been implemented for diagnostics of rare forms of alpha and beta thalassemia. This technique is of additional value in case standard diagnostic tests show a negative result, but a defect is suspected on clinical grounds.

The new light cycler technology for diagnostics in hemochromatosis is not yet ready to replace the current PCR-OLA technique. The prerequisite that the test should take place in a PCR closed system cannot yet be fulfilled. The 'open' variant of the test is incidentally successfully employed when confronted with 'difficult' patient samples. Determination of red blood cell enzymes by means of a new 96-well plate format has been validated and will be implemented in 2007.

New machinery for routine measurement of alpha-thalassemia by means of multiplex PCR has been acquired and validated. The new method, meant to replace the current RFLP technique, will be implemented in 2007.

For better diagnostics of membrane defects we now also include Band 3 protein by means of the flow cytometric EMA (eosin-5-maleimide) labeling test and a PAGE separation of membrane proteins, apart from the common determination of spectrin and acidified glycerol lysis test (AGLT). The EMA test is potentially useful for routine screening of Band 3 protein, the PAGE technique can be used if supplementary research is indicated.

Blood Coagulation

Both a functional assay and an antigen determination assay of protein C are now operational in an automated version.

Experience has been obtained in the rapid diagnosis of thrombotic thrombocytopenic purpura (TTP): about 120 requests, determination of ADAMTS13 and autoantibodies to ADAMTS13. ADAMTS13 is the enzyme that is deficient in TTP. Recently we observed that lupus anticoagulant (LAC) activity caused by antibodies to β 2 glycoprotein I (β 2GPI) correlates strongly with APC resistance. In the past, we found that only a β 2GPI-dependent LAC is correlated with thrombosis. We hypothesize that antibodies to β 2GPI with LAC activity cause APC resistance and that this causes the occurrence of thrombosis in patients with the anti-phospholipid

syndrome. By developing an assay that detects increased APC resistance caused by antiphospholipid antibodies, we should therefore be able to detect antibodies on the basis of pathogenic potential. This concept has been submitted for publication. One of the monoclonal antibodies we selected against the so-called GLA (phospholipids binding) domain of protein S (CLB-PS13) has been proven to block protein S function at several levels (Hackeng et al. Blood 2004; 104:3624-30). This offers excellent possibilities to develop a functional protein S test, based on the action of a unique monoclonal antibody.

Infectious Diseases (blood donor screening)/Viral Diagnostics

In 2006, a start has been made with the validation of the viral screening assays on a new platform, the Architect: HIV combo, HBsAg, anti-HBc, anti-HBs, anti-HCV and anti-Syphilis). Special attention has been paid to the reliability of measuring cadaveric blood samples for safety of tissues. Validation of these assays will be completed in March 2007.

Also in 2006, it has been decided that from July 1, 2008, all blood donations will be screened on a newly established department: the National Sanquin Screening lab (NSS), to be housed in Amsterdam. In order to meet the safety requirements for plasma derivatives, the screening system for Parvovirus B19 was improved in 2006. Recently, new genotypes of the virus were published. The screening system used until 2006 did not recognize the two new molecular variants, genotype 2 and 3. Therefore the test for parvovirus B19 DNA was improved to include detection of these variants. The improved test was evaluated in a study in which plasma for fractionation (n=903) and test pools for screening (n=3502), equivalent to more than 2.6 million donations, were tested in a head to head comparison of the operational test (specific for genotype 1) and the improved test (specific for genotype 1-3). A total of 232 donations revealed a genotype 1 infection with a load above the exclusion limit. One donation with a genotype 2 isolate was detected, with a load above the exclusion limit. Two donations were found with a high load genotype 1 isolate, which were missed in one of the two tests due to a unique mutation(s) in primers or probe. In order to include detection of the rarely found

Parvovirus B19 genotypes 2 and 3 isolates and genotype 1 isolates with a unique mutation, the new operational protocol for screening and 'in process' testing encompasses the combination of both tests evaluated in this study.

In June 2006, screening of plasma-units for fractionation has been expanded with tests for HAV-RNA and HBV-DNA. These tests have been validated according to the European Pharmacopoeia guidelines for this application.

A project was started on the molecular variation in HBV and the consequences thereof for screening using serology and NAT. Screening for the presence of HBV at this moment relies solely on the serological detection of HBsAg. Genetic and subsequent antigenic variation of HBV may compromise the reliability of such screening. We found that among HBV infected Dutch blood donors the variation of HBsAg is much greater than in the classical populations at-risk for HBV. Results obtained by highly sensitive testing of blood donors for presence of HBV DNA with NAT in medium and high endemic regions are surprising. As a partner in a European study we analyzed these samples, mainly derived from Spain. Low levels of HBV DNA are present in some regular donors who have cleared HBV infection in the past and who repeatedly test negative for HBsAg. The HBsAg-genes involved show numerous unique mutations, and the virus may even have become not infectious for blood recipients. Our findings may possibly indicate that a) HBV infection never is cleared completely; b) to avoid eradication HBV ultimately 'sacrifices' its HBsAg; and c) highly sensitive screening for HBV DNA may reveal the presence of HBV variants irrelevant for the safety of the blood supply.

In 2006, the work on detection of bacterial contamination with NAT screening based on 16S ribosomal DNA amplification, should have gone into the next phase for validation in order to be used as a routine screening test for platelets. However, the initially obtained results were difficult to reproduce, because of batch to batch variation in the commercially obtained reagents, making a sufficiently low reproducible background signal impossible. The nucleic acid extraction from gram+ bacteria was also found problematical and badly reproducible. Therefore, it was decided to first try and improve the used methods. We succeeded in a significant

improvement of the procedure for nucleic acid extraction from gram+ bacteria. By choosing a different universal primer and probe set within the 16S rDNA sequence of bacteria, the efficiency of the real time PCR test improved considerably. Together, these improvements result in a test which can detect gram + and (-) bacteria in platelets with a sensitivity between 500 and 1500 CFU/ml. However, batch to batch variation in reagents is still a major cause of concern.

HLA Diagnostics and Paternity testing

The use of Luminex single antigen technology for HLA antibody screening has been investigated. Validation has been finalized and this technology will be introduced early in 2007.

A start has been made with the introduction of killer inhibitory receptor (KIR) genotyping analysis for allogeneic bone marrow transplantation. From reactions of our (clinician) customers, a need for such analysis clearly is present.

During 2006, attention was paid to the following subjects:

- High resolution typing package: further evaluation and validation of the high resolution typing systems for HLA-DQB1, HLA-A and HLA-B;
- Information and Communication Technology (ICT): to reduce administrative errors, results are now directly imported in the laboratory information management system (CLIS) using predefined barcodes. Furthermore, evaluation has started regarding the possibility of converting the outdated 'Eurotransplant (ET) files' in an Oracle based application.

In paternity testing, the introduction of the Q&Q package, which allows sampling of saliva cells at home, has lead to an increased demand.

A start has been made in trying to isolate enough fetal DNA from maternal blood to allow for a reliable DNA analysis which should make it possible to set up antenatal paternity testing.

New platforms

Studies have been started to change the current department structure of Sanquin Diagnostic Services into a structure more based on employed technologies. Apart

from a DNA (isolation) platform, an ELISA platform will be started. Evaluation will be continued during 2007.

TRIX

TRIX (Tranfusion Register on Irregular antibodies and X (cross), a computer-based register for irregular erythrocyte antibodies test problems) has extensively been tested and validated in 2006 and will be nationally implemented in 2007. This register will serve as a tool to prevent (delayed) transfusion reactions in blood recipients, because also only previously detectable irregular erythrocyte antibodies (IEA) will be taken into account by the selection of donor blood.

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Virus Safety Services (VSS) is a virology group dedicated to conducting virus validation studies of plasma-derived products and other biologicals. We offer a range of virus systems, which meet with the latest requirements of national and international regulatory bodies. VSS has more than fifteen years of experience in the field of virus validation. VSS is familiar with blood safety issues being part of a blood-product producing organization.

Virus validation studies

We have a broad experience in validation of various process steps, including the more delicate ones, like column and nanofiltration steps. We have state-of-the art BSL3 facilities, including strict separation between virus negative and virus positive areas. In 1998 full accreditation was granted by the Dutch Council for Accreditation, which participates in the European Cooperation for Accreditation of Laboratories (EAL). An Endorsement of Compliance with the OECD principles of GLP based on assessments performed according to the Netherlands GLP Compliance Monitoring Programme and according to Directive 2004/9/EC was granted in 2005. VSS provides tailor-made solutions for virus validation problems. Detailed information on the virus reducing capacity of process steps is provided. Furthermore smart experimental designs are used for demonstrating robustness of process steps and overall accurate insight into viral safety of your product is achieved.

Virus systems available

Appreciating requirements from relevant guidelines, for performance of virus validation studies VSS can offer the following relevant or model virus systems.

- HIV (Human immunodeficiency virus), a relevant virus for products of human origin
- HAV (Hepatitis A virus), a relevant virus for products of human origin
- Human Parvovirus B19, a relevant virus for products of human origin
- BPV (Bovine parvovirus), a specific model virus for Parvovirus B19
- BVDV (Bovine viral diarrhoea virus), a specific model virus for hepatitis C virus
- CPV (Canine parvovirus), a specific model virus for Parvovirus B19
- EMC (Encephalomyocarditis virus), a specific model virus for hepatitis A virus

- PPV (Porcine parvovirus), a specific model virus for Parvovirus B19
- PSR (Pseudorabies virus), a general model virus for lipid enveloped DNA viruses (e.g. hepatitis B virus)
- SV40 (Simian virus 40), a general model virus for non-enveloped DNA viruses
- TGEV (Transmissible gastroenteritis virus), a specific model virus for SARS (severe acute respiratory syndrome)
 - VSV (Vesicular stomatitis virus), a general model for lipid enveloped RNA viruses

Research lines

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 - Validation of disinfection procedures 70

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The mission of Sanquin Consultancy Services (SCS) is to provide guidance and advice services to restricted economy countries.

Objectives are

- (I) to support restricted economy countries in developing safe, efficacious and sustainable blood supply systems based on current quality principles,
- (II) to provide modular training programmes on transfusion medicine for restricted economy countries focused on the managerial and quality aspects of the transfusion chain, and
- (III) to extend and strengthen the training and consultative potential within the Sanquin organization.

In close collaboration with the Academic Institute for International Development of Transfusion Medicine (IDTM) educational programmes and applied research in health sciences related to the field of transfusion medicine have been developed. Existing educational and scientific collaborations of the Academic Institute IDTM and SCS at the University of Groningen have contributed to the development.

Educational programmes

- 1 Noordelijke Hogeschool Leeuwarden (NHL)
 - a Three BBA students from China worked on a SCS/IDTM marketing programme for China;
 - b Two BBA students from China worked on the development of an SCS and IDTM brochure and website (SCS Communication plan).
- 2 Vrije Universiteit (VU) Amsterdam, Dept Biology and Society, Faculty of Earth and Life Sciences in collaboration with the Dept of Social Pharmacy, Pharmaco-Epidemiology and Pharmacotherapy, Faculty of Pharmacy, RUG.
 - a Graduation student working on safety of the blood supply; an attempt to predict the value of the current routine TTI marker screening in Kampala, Uganda.

- 3 Groningen University (RUG), Faculty of Mathematics and Natural Sciences and Dept of Social Pharmacy, Pharmaco-Epidemiology and Pharmacotherapy, Faculty of Pharmacy.
a Graduation student in Sciences, Policy and Business, working on Blood transfusion policy and practice at Mulago Hospital in Kampala, Uganda.

Scientific projects

- 1 University of Amsterdam (UvA), Faculty of Economy and Econometry, Dept of Operations Research.
a PhD fellow working on a formal approach for practical optimization of blood platelet production.
- 2 Groningen University (RUG), Dept of Social Pharmacy, Pharmaco-Epidemiology and Pharmacotherapy, Faculty of Pharmacy.
a PhD fellow working on Health Economics of blood transfusion safety in developing countries.
- 3 Groningen University (RUG), Faculty of Medical Sciences and Makerere University, Faculty of Medicine, Kampala, Uganda.
a PhD fellow from Kampala, Uganda working on 'autologous and homologous blood transfusion: indications, safety, accurate determination of blood requirements and cost'.

Patent port folio and licensing

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Besides results of research projects published in scientific and professional publications, Sanquin also disseminates knowledge in the form of patents and other forms of Intellectual Property. As Sanquin is not only a research institute, but first and foremost a blood supply organization, product and process innovation is also very important. Not only for Sanquin activities itself, but also for biotech and pharmaceutical companies and third parties active in the R&D field of devices used by blood banks in blood processing.

In recent years it has become apparent that monoclonal antibodies, as therapeutic biologicals, seem to offer new possibilities for patients that were difficult to treat otherwise; for instance patients with autoimmune diseases or cancer.

In the table on the next page you will find an overview of the valorization status of Sanquin Research patents and hybridoma's of the last five years. Most patents/ hybridomas listed have a primary therapeutic application.

Sanquin patents 2001-2006	Sanquin hybridoma's 2001-2006	Status
MHC Multimeren		Open for licensing
Diagnostic methods involving determining gene copy numbers		Open for licensing
DCs maturation		Open for licensing
CD 97		3 rd party assigned
	CD70	3 rd party licensed
	B9	3 rd party licensed
Trombose PCR		3 rd party licensed
Anti-FVIII		Open for licensing
TAC-TAT fusion peptides		Terminated
	2C8	Option to license
Anti C3-2	Anti C3-2	C3-2 patent terminated
Anti c-1q	Anti c-1q	Patent terminated
C1-est inhibitor in AMI		3 rd party licensed
1E4	1E4	Option to license
	CD17	3 rd party licensed
	4-7B	3 rd party licensed
FVIII mutants		Open for licensing
Mabs for intact haemostatic proteins		Open for licensing
	Anti IL 6	3 rd party licensed

Sponsors

Various organizations, charities and industries have contributed towards research of Sanquin by funding investigators, travel expenses, equipment or offering free materials:

Landsteiner Laboratory

Sanquin Research and the Academic Medical Center of the University of Amsterdam collaborate in the joint AMCSanquin Landsteiner Laboratory for Blood Transfusion Research, housed in Sanquin's premises in Amsterdam.

2nd source of funding

Dutch Medical Research Council (ZON/MW)
Netherlands Genomics Initiative (NROG)
Netherlands Organization for Scientific Research (NWO)
European Commission

3rd source of funding (Charities, private funding Organizations, non-Dutch Research councils)

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Chronic Granulomatosis Disease Trust
Deutsche Forschungsgemeinschaft
Dutch AIDS Fund (SAF)
Dutch Cancer Fund /KWF
Dutch Cancer Society
Dutch Heart Foundation
Dutch Thrombosis Foundation
Foundation Jan Kornelis de Cock
Foundation for Pediatric Cancer Research
Friends of Research on MS
Gratama Stichting
Joghem van Loghem Foundation

Landsteiner Foundation for Blood Research (LSBR)
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Municipal Health Services Amsterdam (GG&GD)
National AIDS Therapy Evaluation Center
National Foundation for Rheumatism
Nefkens Foundation
Netherlands Asthma Foundation
Platform Alternatieve Dierproeven
Princess Beatrix Foundation
SENER/Novem
Stichting Fondsenwervingsacties Volksgezondheid
Tekke Huizinga Foundation

4th source of funding: Contract and codevelopment partners

Ablynx
Academic Hospital, University of Maastricht
Academic Medical Center, University of Amsterdam
Adenbrooks Hospital
American Red Cross
Amcell Corporation
Amgen
ASAC
A-Viral ASA
Baxter BioScience
Baxter Health Care
Baxter Oncology
BCSI
Berna Biotech
Biogen
BioMérieux Nederland

BioSafe
Biotest Pharma GmbH
Boehringer Ingelheim Pharmaceuticals Inc.
Cardiovascular Research Institute Maastricht (CARIM)
Centocor
Cerus Corporation
Chiron corporation
Crucell
Diacclone
DSM Biologics
Finnish Red Cross
Fresenius HemoCare
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Natal Bioproducts Institute
Navigant Bonville
Nefkens
NIZO laboratories
Numico
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Organon/Schering Plough
Ortho-Clinical Diagnostics Pharming
Philips
PhotoBioChem
ProLacta
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Synaps BV
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Universiteit Amsterdam
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Vitaleech Bioscience
Vrije Universiteit Medical Center, Amsterdam
Wageningen University and Research Center
Zentech s.a.
Zentral Laborator Bern

Other sources of funding
Ministry of Economic Affairs (WBSO)

Publications

Papers in international journals	157
Miscellaneous publications	169

Papers in international journals

On our website www.sanquinresearch.nl all our publications are listed in a searchable database. Where available, links to PubMed abstracts are included on that website.

Alphabetically by first author

Aalberse RC, Stadler BM. In silico predictability of allergenicity: From amino acid sequence via 3-D structure to allergenicity. *Mol Nutr Food Res* 2006; 50:625-7.

Akkerdaas JH, Schocker F, Vieths S, Versteeg S, Zuidmeer L, Hefle SL, Aalberse RC, Richter K, Ferreira F, van Ree R. Cloning of oleosin, a putative new hazelnut allergen, using a hazelnut cDNA library. *Mol Nutr Food Res* 2006; 50(1):18-23.

Allain JP, Reesink HW, Lucey C. A European perspective on the management of donors and units testing positive for hepatitis B virus DNA. *Transfusion* 2006; 46 (7):1256-8.

Antens CJ, Oldenwening M, Wolse A, Gehring U, Smit HA, Aalberse RC, Kerkhof M, Gerritsen J, de Jongste JC, Brunekreef B. Repeated measurements of mite and pet allergen levels in house dust over a time period of 8 years. *Clin Exp Allergy* 2006; 36(12):1525-31.

Badlou BA, Wu YP, Smid WM, Akkerman JW. Platelet binding and phagocytosis by macrophages. *Transfusion* 2006; 46(8):1432-43.

Badlou BA, Wu YP, Spierenburg G, Smid WM, Akkerman JW. Role of glycoprotein Ibalpha in phagocytosis of platelets by macrophages *Transfusion* 2006; 46(12):2090-9.

Baidoshvili A, Krijnen PA, Kupreishvili K, Ciurana C, Bleeker W, Nijmeijer R, Visser CA, Visser FC, Meijer CJ, Stooker W, Eijssman L, van Hinsbergh VW, Hack CE, Niessen HW, Schalkwijk CG. N ϵ -(Carboxymethyl)lysine Depositions in Intramyocardial Blood Vessels in Human and Rat Acute Myocardial Infarction. A Predictor or Reflection of Infarction? *Arterioscler Thromb Vasc Biol* 2006; 26(11):2497-503.

Bakema JE, de Haij S, den Hartog-Jager CF, Bakker J, Vidarsson G, van Egmond M, van de Winkel JG, Leusen JH. Signaling through mutants of the IgA receptor CD89 and consequences for Fc receptor gamma-chain interaction. *J Immunol* 2006; 176(6):3603-10.

Bartelds GM, Wolbink GJ, Stapel S, Aarden L, Lems WF, Dijkmans BA, Nurmohamed MT. High levels of human anti-human antibodies to adalimumab in a patient not responding to adalimumab treatment. *Ann Rheum Dis* 2006; 65(9):1249-50.

Baumgarten R, van Gelder W, van Wintershoven J, Maaskant-van Wijk PA, Beckers EA. Recurrent acute hemolytic transfusion reactions by antibodies against Do antigens, not detected by cross-matching. *Transfusion* 2006; 46(2):244-9.

- Bekker V, Scherpbier H, Beld M, Piriou E, Breda A, Lange J, Leth F, Jurriaans S, Alders S, Dillen PW, Baarle D, Kuijpers T. Epstein-Barr Virus Infects B and Non-B Lymphocytes in HIV-1-Infected Children and Adolescents. *J Infect Dis* 2006; 194(9):1323-30.
- Bekker V, Westerlaken GH, Scherpbier H, Alders S, Zaaijer H, van Baarle D, Kuijpers T. Varicella vaccination in HIV-1-infected children after immune reconstitution. *AIDS* 2006; 20(18):2321-9.
- Biezeveld MH, Geissler J, Weverling GJ, Kuipers IM, Lam J, Ottenkamp J, Kuijpers TW. Polymorphisms in the mannose-binding lectin gene as determinants of age-defined risk of coronary artery lesions in Kawasaki disease. *Arthritis Rheum* 2006; 54(1):369-76.
- Blaak H, van der Ende ME, Boers PH, Schuitemaker H, Osterhaus AD. *In vitro* replication capacity of HIV-2 variants from long-term aviremic individuals. *Virology* 2006; 353:144-54.
- Bouma BN, van Mourik JA. Unraveling the mystery of von Willebrand factor. *J Thromb Haemost* 2006; 4(3):489-95.
- Bouman CS, de Pont AC, Meijers JC, Bakhtiari K, Roem D, Zeerleder S, Wolbink G, Korevaar JC, Levi M, de Jonge E. The effects of continuous venovenous hemofiltration on coagulation activation. *Crit Care* 2006; 10(5):R150.
- Bovenschen N, van Stempvoort G, Voorberg J, Mertens K, Meijer AB. Proteolytic cleavage of factor VIII heavy chain is required to expose the binding-site for low-density lipoprotein receptor-related protein within the A2 domain. *J Thromb Haemost* 2006; 4(7):1487-93.
- Brand A, Novotny V, Tomson B. Platelet transfusion therapy: from 1973 to 2005. *Hum Immunol* 2006; 67(6):413-8.
- Breunis WB, Biezeveld MH, Geissler J, Ottenkamp J, Kuipers IM, Lam J, Hutchinson A, Welch R, Chanock SJ, Kuijpers TW. Vascular endothelial growth factor gene haplotypes in Kawasaki disease. *Arthritis Rheum* 2006; 54(5):1588-94.
- Bril WS, van Helden PM, Hausl C, Zuurveld MG, Ahmad RU, Hollestelle MJ, Reitsma PH, Fijnvandraat K, van Lier RA, Schwarz HP, Mertens K, Reijpert BM, Voorberg J. Tolerance to factor VIII in a transgenic mouse expressing human factor VIII cDNA carrying an Arg(593) to Cys substitution. *Thromb Haemost* 2006; 95(2):341-7.
- Brouwer N, Dolman KM, van Zwieten R, Nieuwenhuys E, Hart M, Aarden LA, Roos D, Kuijpers TW. Mannan-binding lectin (MBL)-mediated opsonization is enhanced by the alternative pathway amplification loop. *Mol Immunol* 2006; 43(13):2051-60.
- Bultink IE, Hamann D, Seelen MA, Hart MH, Dijkmans BA, Daha MR, Voskuyl AE. Deficiency of functional mannose-binding lectin is not associated with infections in patients with systemic lupus erythematosus (SLE). *Arthritis Res Ther* 2006; 8(6):R183.

- Bunnik EM, Quakkelaar ED, van Nuenen AC, Boeser-Nunnink B, Schuitemaker H. Increased neutralization sensitivity of recently emerged CXCR4-using HIV-1 as compared to co-existing CCR5-using variants from the same patient. *J Virol* 2006; 81(2):525-31.
- Caillet-Fauquet P, Di Giambattista M, de Launoit Y, Laub R. Does quantification of virus transcription provide a real measure of parvovirus B19 infectivity? *J Clin Virol* 2006; 37:77-8.
- Cauwenberghs S, Feijge MA, Theunissen E, Heemskerck JW, van Pampus EC, Curvers J. Novel methodology for assessment of prophylactic platelet transfusion therapy by measuring increased thrombus formation and thrombin generation. *Br J Haematol* 2006; 136(3):480-90.
- Ciurana CL, Hack CE. Competitive binding of pentraxins and IgM to newly exposed epitopes on late apoptotic cells. *Cell Immunol* 2006; 239(1):14-21.
- Curvers J, Groen P. Automatisering van de unit Productie binnen de bloedbanken. *NVB bulletin* 2006; 1: 2-4.
- Da Costa Martins P, van Gils JM, Mol A, Hordijk PL, Zwaginga JJ. Platelet binding to monocytes increases the adhesive properties of monocytes by up-regulating the expression and functionality of β 1 and β 2 integrins. *J Leukoc Biol* 2006; 79:499-507.
- De Boer HC, Verseyden C, Ulfman LH, Zwaginga JJ, Bot I, Biessen EA, Rabelink TJ, Van Zonneveld AJ. Fibrin and Activated Platelets Cooperatively Guide Stem Cells to a Vascular Injury and Promote Differentiation Towards an Endothelial Cell Phenotype. *Arterioscler Thromb Vasc Biol* 2006; 26(7):1653-9.
- De Haas M, van der Schoot CE, Beiboer SH, Feskens M, Cheroutre G, Maaskant-van Wijk PA. Red blood cell and platelet genotyping: from current practice to future high-throughput donor typing. *Transfus Med Hemother* 2006; 33:260-6.
- De Korte D, Curvers J, De Kort WL, Hoekstra T, van der Poel CL, Beckers EA, Marcelis JH. Effects of skin disinfection method, deviation bag, and bacterial screening on clinical safety of platelet transfusions in the Netherlands. *Transfusion* 2006; 46(3):476-85.
- Dekker RJ, Boon RA, Rondaij MG, Kragt A, Volger OL, Elderkamp YW, Meijers JC, Voorberg J, Pannekoek H, Horrevoets AJ. KLF2 provokes a gene expression pattern that establishes functional quiescent differentiation of the endothelium. *Blood* 2006; 107(11):4354-63.
- Dijkstra-Tiekstra MJ, van der Schoot CE, Pietersz RN, Reesink HW. Response to C. Navarette and M. Contreras. *Vox Sang* 2006; 90 (3):206.

Dohmen SE, Verhagen OJ, de Groot SM, Stott LM, Aalberse RC, Urbaniak SJ, van der Schoot CE. The analysis and quantification of a clonal B cell response in a hyperimmunized anti-D donor. *Clin Exp Immunol* 2006; 144(2):223-32.

Dohmen SE, Verhagen OJ, Muijt J, Ligthart PC, van der Schoot CE. The restricted use of IGHV3 superspecies genes in anti-Rh is not limited to hyperimmunized anti-D donors. *Transfusion* 2006; 46(12):2162-8.

Drayer AL, Olthof SG, Vellenga E. Mammalian target of rapamycin is required for thrombopoietin-induced proliferation of megakaryocyte progenitors. *Stem Cells* 2006; 24(1):105-14 (already listed in 2005 as E-pub ahead of print).

Fernandez-Rivas M, Bolhaar S, Gonzalez-Mancebo E, Asero R, van Leeuwen A, Bohle B, Ma Y, Ebner C, Rigby N, Sancho AI, Miles S, Zuidmeer L, Knulst A, Breiteneder H, Mills C, Hoffmann-Sommergruber K, van Ree R. Apple allergy across Europe: how allergen sensitization profiles determine the clinical expression of allergies to plant foods. *J Allergy Clin Immunol* 2006; 118(2):481-8.

Frakking FN, Brouwer N, Zweers D, Merkus MP, Kuijpers TW, Offringa M, Dolman KM. High prevalence of mannose-binding lectin (MBL) deficiency in premature neonates. *Clin Exp Immunol* 2006; 145(1):5-12.

Frakking FN, van de Wetering MD, Brouwer N, Dolman KM, Geissler J, Lemkes B, Caron HN, Kuijpers TW. The role of mannose-binding lectin (MBL) in paediatric oncology patients with febrile neutropenia. *Eur J Cancer* 2006; 42(7):909-16.

Fribourg C, Meijer AB, Mertens K. The Interface between the EGF2 Domain and the Protease Domain in Blood Coagulation Factor IX Contributes to Factor VIII Binding and Factor X Activation. *Biochemistry* 2006; 45(35):10777-85.

Frijns CJ, Fijnheer R, Algra A, van Mourik JA, van Gijn J, Rinkel GJ. Early circulating levels of endothelial cell activation markers in aneurysmal subarachnoid haemorrhage: associations with cerebral ischaemic events and outcome. *J Neurol Neurosurg Psychiatry* 2006; 77(1):77-83.

Geels MJ, Jansen CA, Baan E, De Cuyper IM, van Schijndel GJ, Schuitemaker H, Goudsmit J, Pollakis G, Miedema F, Paxton WA, van Baarle D. CTL escape and increased viremia irrespective of HIV-specific CD4+ T-helper responses in two HIV-infected individuals. *Virology* 2006; 345(1):209-19.

Grootkerk-Tax MG, Ait Soussan A, de Haas M, Maaskant-van Wijk PA, van der Schoot CE. Evaluation of prenatal RHD typing strategies on cell-free fetal DNA from maternal plasma. *Transfusion* 2006; 46(12):2142-8.

Grootkerk-Tax MG, van Wintershoven JD, Ligthart PC, van Rhenen DJ, van der Schoot CE, Maaskant-van Wijk PA. RHD(T201R, F223V) cluster analysis in five different ethnic groups and serologic characterization of a new Ethiopian variant DARE, the DIII type 6, and the RHD(F223V). *Transfusion* 2006; 46(4):606-15.

Haanen JBAG, Baars A, Gomez R, Weder P, Smits M, De Gruijl TD, von Blumberg BME, Bloemena E, Scheper RJ, van Ham SM, Pinedo HM, van den Eertwegh AJM. Melanoma-specific Tumor-Infiltrating Lymphocytes predict survival in vaccinated advanced-stage melanoma patients. *Canc Immunol Immunother* 2006; 55:451.

Heijnen BH, Straatsburg IH, Diaz Padilla N, van Mierlo GJ, Hack CE, van Gulik TM. Inhibition of classical complement activation attenuates liver ischaemia and reperfusion injury in a rat model. *Clin Exp Immunol* 2006; 143(1):15-23.

Hollestelle MJ, Donkor C, Mantey EA, Chakravorty SJ, Craig A, Osei Akoto A, O'donnell J, van Mourik JA, Bunn J. Von Willebrand factor propeptide in malaria: evidence of acute endothelial cell activation. *Br J Haematol* 2006; 133(5):562-9.

Hordijk PL. Endothelial signalling events during leukocyte transmigration. *FEBS J* 2006; 273:4408-15.

Hordijk PL. Regulation of NADPH oxidases: the role of Rac proteins. *Circ Res* 2006; 98(4):453-62.

Jansen CA, De Cuyper IM, Hooibrink B, van der Bij AK, van Baarle D, Miedema F. Prognostic value of HIV-1 Gag-specific CD4+ T-cell responses for progression to AIDS analyzed in a prospective cohort study. *Blood* 2006; 107(4):1427-33.

Jansen CA, Piriou E, De Cuyper IM, van Dort K, Lange JM, Miedema F, van Baarle D. Long-term highly active antiretroviral therapy in chronic HIV-1 infection: evidence for reconstitution of antiviral immunity. *Antivir Ther* 2006; 11(1):105-16.

Jansen CA, van Baarle D, Miedema F. HIV-specific CD4+ T cells and viremia: who's in control? *Trends Immunol* 2006; 27(3):119-24.

Jansen M, Brand A, von Lindern JS, Scherjon S, Walther FJ. Potential use of autologous umbilical cord blood red blood cells for early transfusion needs of premature infants. *Transfusion* 2006; 46(6):1049-56.

Janssen MP, van der Poel CL, Buskens E, Bonneux L, Bonsel GJ, van Hout BA. Costs and benefits of bacterial culturing and pathogen reduction in the Netherlands. *Transfusion* 2006; 46(6):956-65.

Kamerbeek NM, van Zwielen R, de Boer M, Morren G, Vuil H, Bannink N, Lincke C, Dolman KM, Becker K, Schirmer RH, Gromer S, Roos D. Molecular basis of glutathione reductase deficiency in human blood cells. *Blood* 2006; 109(8):3560-6.

Keller TT, Choi D, Nagel C, te Velthuis H, Gerdes VE, Wareham NJ, Bingham SA, Luben R, Hack CE, Reitsma PH, Levi M, Khaw KT, Boekholdt SM. Tissue factor serum levels and the risk of future coronary artery disease in apparently healthy men and women: the EPIC-Norfolk prospective population study. *J Thromb Haemost* 2006; 4(11):2391-6.

Kerkhoffs JL, Eikenboom JC, Schipperus MS, Wordragen-Vlaswinkel RJ, Brand R, Harvey MS, de Vries RR, Barge R, van Rhenen DJ, Brand A. A multicenter randomized study of the efficacy of transfusions with platelets stored in platelet additive solution II versus plasma. *Blood* 2006; 108(9):3210-5.

Keuren JF, Magdeleyns EJ, Govers-Riemslog JW, Lindhout T, Curvers J. Effects of storage-induced platelet microparticles on the initiation and propagation phase of blood coagulation. *Br J Haematol* 2006; 134(3):307-13.

Koker MY, Sanal O, de Boer M, Tezcan I, Metin A, Tan C, Ersoy F, Roos D. Skewing of X-chromosome inactivation in three generations of carriers with X-linked chronic granulomatous disease within one family. *Eur J Clin Invest* 2006; 36(4):257-64.

Koppelman MH, Sjerps MS, de Waal M, Reesink HW, Cuypers HT. No evidence of West Nile virus infection in Dutch blood donors. *Vox Sang* 2006; 90(3):166-9.

Krol A, Lensen R, Veenstra J, Prins M, Schuitemaker H, Coutinho RA. Impact of CCR5 Delta32/+ Deletion on Herpes Zoster Among HIV-1-Infected Homosexual Men. *Eur J Epidemiol* 2006; 21(6):469-73.

Kuijpers TW, van Bruggen R, Kamerbeek N, Tool AT, Hicsonmez G, Gurgey A, Karow A, Verhoeven AJ, Seeger K, Sanal O, Niemeyer C, Roos D. Natural history and early diagnosis of LAD-1/variant syndrome. *Blood* 2006; 109(8):3529-37.

Levi M, Choi G, Picavet C, Hack CE. Self-administration of C1-inhibitor concentrate in patients with hereditary or acquired angioedema caused by C1-inhibitor deficiency. *J Allergy Clin Immunol* 2006; 117(4):904-8.

Lorenowicz MJ, van Gils J, de Boer M, Hordijk PL, Fernandez-Borja M. Epac1-Rap1 signaling regulates monocyte adhesion and chemotaxis. *J Leukoc Biol* 2006; 80:1542-52.

Luken BM, Kaijen PH, Turenhout EA, Hovinga JA, van Mourik JA, Fijnheer R, Voorberg J. Multiple B-cell clones producing antibodies directed to the spacer and disintegrin/thrombospondin type-1 repeat 1 (TSP1) of ADAMTS13 in a patient with acquired thrombotic thrombocytopenic purpura. *J Thromb Haemost* 2006; 4(11):2355-64.

Luken BM, Turenhout EA, Kaijen PH, Greuter MJ, Pos W, van Mourik JA, Fijnheer R, Voorberg J. Amino acid regions 572-579 and 657-666 of the spacer domain of ADAMTS13 provide a common antigenic core required for binding of antibodies in patients with acquired TTP. *Thromb Haemost* 2006; 96(3):295-301.

Martens DH, Kuijpers TW, Maianski NA, Rake JP, Smit GP, Visser G. A patient with common glycogen storage disease type Ib mutations without neutropenia or neutrophil dysfunction. *J Inher Metab Dis* 2006; 29(1):224-5.

Matsui EC, Diette GB, Krop EJ, Aalberse RC, Smith AL, Eggleston PA. Mouse allergen-specific immunoglobulin G4 and risk of mouse skin test sensitivity. *Clin Exp Allergy* 2006; 36(8):1097-103.

McGrath FD, Brouwer MC, Arlaud GJ, Daha MR, Hack CE, Roos A. Evidence That Complement Protein C1q Interacts with C-Reactive Protein through Its Globular Head Region. *J Immunol* 2006; 176(5):2950-7.

Meischl C, Krijnen PA, Sipkens JA, Cillessen SA, Munoz IG, Okroj M, Ramska M, Muller A, Visser CA, Musters RJ, Simonides WS, Hack CE, Roos D, Niessen HW. Ischemia induces nuclear NOX2 expression in cardiomyocytes and subsequently activates apoptosis. *Apoptosis* 2006; 11(6):913-21.

Moroff G, Eichler H, Brand A, Kekomaki R, Kurtz J, Letowska M, Pamphilon D, Read EJ, Porretti L, Lecchi L, Reems JA, Sacher R, Seetharaman S, Takahashi TA. Biomedical Excellence for Safer Transfusion (BEST) Collaborative. Multiple-laboratory comparison of *in vitro* assays utilized to characterize hematopoietic cells in cord blood. *Transfusion* 2006; 46(4):507-15.

Nathwani AC, Gray JT, Ng CY, Zhou J, Spence Y, Waddington SN, Tuddenham EG, Kemball-Cook G, McIntosh J, Boon-Spijker M, Mertens K, Davidoff AM. Self complementary adeno-associated virus vectors containing a novel liver-specific human factor IX expression cassette enable highly efficient transduction of murine and nonhuman primate liver. *Blood* 2006; 107(7):2653-61.

Newland A, Caulier MT, Kappers-Klunne M, Schipperus MR, Lefrere F, Zwaginga JJ, Christal J, Chen CF, Nichol JL. An open-label, unit dose-finding study of AMG 531, a novel thrombopoiesis-stimulating peptibody, in patients with immune thrombocytopenic purpura. *Br J Haematol* 2006; 135(4):547-53.

Nielen MM, van Schaardenburg D, Lems WF, van de Stadt RJ, de Koning MH, Reesink HW, Habibuw MR, van der Horst-Bruinsma IE, Twisk JW, Dijkmans BA. Vitamin D deficiency does not increase the risk of rheumatoid arthritis: Comment on the article by Merlino et al. *Arthritis Rheum* 2006; 54 (11):3719-20.

Nielen MM, van Schaardenburg D, Reesink HW, Twisk JW, van de Stadt RJ, van der Horst-Bruinsma IE, de Koning MH, Habibuw MR, Dijkmans BA. Simultaneous development of acute phase response and autoantibodies in preclinical rheumatoid arthritis. *Ann Rheum Dis* 2006; 65(4):535-7.

Nossent AY, van Marion V, van Tilburg NH, Rosendaal FR, Bertina RM, van Mourik JA, Eikenboom HC. Von Willebrand factor and its propeptide: the influence of secretion and clearance on protein levels and the risk of venous thrombosis. *J Thromb Haemost* 2006; 4(12):2556-62.

Oedayrajsingh-Varma M, van Ham S, Knippenberg M, Helder M, Klein-Nulend J, Schouten T, Ritt M, van Milligen F. Adipose tissue-derived mesenchymal stem cell yield and growth characteristics are affected by the tissue-harvesting procedure. *Cytotherapy* 2006; 8(2):166-77.

Page-Christiaens GC, Bossers B, van der Schoot CE, de Haas M. Use of Bi-Allelic Insertion/Deletion Polymorphisms as a Positive Control for Fetal Genotyping in Maternal Blood: First Clinical Experience. *Ann N Y Acad Sci* 2006; 1075:123-9.

Piriou ER, van Dort K, Weel JF, Bemelman FJ, Gamadia LE, van Oers MH, van Baarle D. Detailed kinetics of EBV-specific CD4+ and CD8+ T cells during primary EBV infection in a kidney transplant patient. *Clin Immunol* 2006; 119(1):16-20.

Reesink HW, Allain JP. Management of donors and blood products reactive for hepatitis B virus DNA. *Vox Sang* 2006; 91(4):281

Reesink HW, Mohammadi T, Pietersz RNI, Savelkoul HM. Applications of real-time PCR in the screening of platelet concentrates for bacterial contamination. *Expert Rev Mol Diagn* 2006; 6(6):865-72.

Remijn JA, da Costa Martins P, IJsseldijk MJ, Sixma JJ, de Groot PG, Zwaginga JJ. Impaired platelet adhesion to lysed fibrin, whereas neutrophil adhesion remains intact under conditions of flow. *Blood Coagul Fibrinolysis* 2006; 17(5):421-4.

Reumaux D, Hordijk PL, Duthilleul P, Roos D. Priming by tumor necrosis factor- α of human neutrophil NADPH-oxidase activity induced by antiproteinase-3 or antimyeloperoxidase antibodies. *J Leukoc Biol* 2006; 80:1424-33.

Rondaj MG, Bierings R, Kragt A, Gijzen KA, Sellink E, van Mourik JA, Fernandez-Borja M, Voorberg J. Dynein-Dynactin Complex Mediates Protein Kinase A-Dependent Clustering of Weibel-Palade Bodies in Endothelial Cells. *Arterioscler Thromb Vasc Biol* 2006; 26(1):49-55.

Rondaj MG, Bierings R, Kragt A, van Mourik JA, Voorberg J. Dynamics and Plasticity of Weibel-Palade Bodies in Endothelial Cells. *Arterioscler Thromb Vasc Biol* 2006; 26:1002-7.

Roos D, de Boer M, Koker MY, Dekker J, Singh-Gupta V, Ahlin A, Palmblad J, Sanal O, Kurenko-Dept, Jolles S, Wolach B. Chronic granulomatous disease caused by mutations other than the common GT deletion in NCF1, the gene encoding the p47(phox) component of the phagocyte NADPH oxidase. *Hum Mutat* 2006; 27(12):1218-29.

- Schonewille H, van de Watering LM, Brand A. Additional red blood cell alloantibodies after blood transfusions in a nonhematologic alloimmunized patient cohort: is it time to take precautionary measures? *Transfusion* 2006; 46(4):630-5.
- Schonewille H, van de Watering LM, Loomans DS, Brand A. Red blood cell alloantibodies after transfusion: factors influencing incidence and specificity. *Transfusion* 2006; 46(2):250-6.
- Sharon RF, Bierings M, Vrieling H, Versluys B, Boelens JJ. Pre-emptive granulocyte transfusions enable allogeneic hematopoietic stem cell transplantation in pediatric patients with chronic infections. *Bone Marrow Transplant* 2006; 37(3):331-3.
- Smit LA, van Maldegem F, Langerak AW, van der Schoot CE, de Wit MJ, Bea S, Campo E, Bende RJ, van Noesel CJ. Antigen receptors and somatic hypermutation in B-cell chronic lymphocytic leukemia with Richter's transformation. *Haematologica* 2006; 91(7):903-11.
- So-Osman C, Nelissen RG, Eikenboom HC, Brand A. Efficacy, safety and user-friendliness of two devices for postoperative autologous shed red blood cell re-infusion in elective orthopaedic surgery patients: A randomized pilot study. *Transfus Med* 2006; 16(5):321-8.
- Sprong T, Roos D, Weemaes C, Neeleman C, Geesing CL, Mollnes TE, van Deuren M. Deficient alternative complement pathway activation due to factor D deficiency by two novel mutations in the Complement Factor D gene in a family with meningococcal infections. *Blood* 2006; 107(12):4865-70.
- Ten Brinke A, Karsten ML, Dieker MC, Zwaginga JJ, Vrieling H, van Ham M. Generation of dendritic cells for immunotherapy is minimally impaired by granulocytes in the monocyte preparation. *Immunobiology* 2006; 211(6-8):633-40.
- Ten Klooster JP, Evers EE, Janssen L, Machesky LM, Michiels F, Hordijk P, Collard JG. Interaction between Tiam1 and the Arp2/3 complex links activation of Rac to actin polymerization. *Biochem J* 2006; 397(1):39-45.
- Ten Klooster JP, Jaffer ZM, Chernoff J, Hordijk PL. Targeting and activation of Rac1 are mediated by the exchange factor {beta}-Pix. *J Cell Biol* 2006; 172(5):759-69.
- Terpstra FG, Parkkinen J, Tölö H, Koenderman AH, ter Hart HG, von Bonsdorff L, Törmä E, van Engelenburg FA. Viral safety of Nanogam(R), a new 15 nm-filtered liquid immunoglobulin product. *Vox Sang* 2006; 90(1):21-32.
- Thorpe SJ, Fox B, Heath AB, Scott M, de Haas M, Kochman S, Padilla A. International standards for minimum potency of anti-A and anti-B blood grouping reagents: evaluation of candidate preparations in an international collaborative study. *Vox Sang* 2006; 91(4):336-44.
- Thorpe SJ, Fox B, Heath AB, Scott M, de Haas M, Kochman S, Padilla A. An International Standard for specifying the minimum potency of anti-D blood-grouping reagents: evaluation of a candidate preparation in an international collaborative study. *Vox Sang* 2006; 90(2):131-9.

- Tijssen MR, van der Schoot CE, Voermans C, Zwaginga JJ. Clinical approaches involving thrombopoietin to shorten the period of thrombocytopenia after high-dose chemotherapy. *Transfus Med Rev* 2006; 20(4):283-93.
- Tjernberg P, Vos HL, Spaargaren-van Riel CC, Luken BM, Voorberg J, Bertina RM, Eikenboom JC. Differential effects of the loss of intrachain- versus interchain-disulfide bonds in the cystine-knot domain of von Willebrand factor on the clinical phenotype of von Willebrand disease. *Thromb Haemost* 2006; 96(6):717-24.
- Trannoy LL, Terpstra FG, de Korte D, Lagerberg JW, Verhoeven AJ, Brand A, van Engelenburg FA. Differential sensitivities of pathogens in red cell concentrates to Tri-P(4)-photoinactivation. *Vox Sang* 2006; 91(2):111-8.
- Tschopp CM, Spiegl N, Didichenko S, Lutmann W, Julius P, Virchow JC, Hack CE, Dahinden CA. Granzyme B, a novel mediator of allergic inflammation: Its induction and release in blood basophils and human asthma. *Blood* 2006; 108(7):2290.
- Uss E, Rowshani AT, Hooibrink B, Lardy NM, van Lier RA, ten Berge IJ. CD103 is a marker for alloantigen-induced regulatory CD8+ T cells. *J Immunol* 2006; 177(5):2775-83.
- Van Aart L, Eijkhout HW, Kamphuis JS, Dam M, Schattenkerk ME, Schouten TJ, Ploeger B, Strengers PF. Individualized dosing regimen for prothrombin complex concentrate more effective than standard treatment in the reversal of oral anticoagulant therapy: An open, prospective randomized controlled trial. *Thromb Res* 2006; 118:313-20.
- Van Berkel WJ, Kamerbeek NM, Fraaije MW. Flavoprotein monooxygenases, a diverse class of oxidative biocatalysts. *J Biotechnol* 2006; 124(4):670-89.
- Van de Laar TJ, Koppelman MH, van der Bij AK, Zaaijer HL, Cuijpers HT, van der Poel CL, Coutinho RA, Bruisten SM. Diversity and origin of hepatitis C virus infection among unpaid blood donors in the Netherlands. *Transfusion* 2006; 46(10):1719-28.
- Van de Watering L, Lorinser J, Versteegh M, Westendorp R, Brand A. Effects of storage time of red blood cell transfusions on the prognosis of coronary artery bypass graft patients. *Transfusion* 2006; 46(10):1712-8.
- Van der Bij AK, Coutinho RA, van der Poel CL. Surveillance of risk profiles among new and repeat blood donors with transfusion-transmissible infections from 1995 through 2003 in the Netherlands. *Transfusion* 2006; 46(10):1729-36.
- Van der Meer PF, de Wildt-Eggen J. The effect of whole-blood storage time on the number of white cells and platelets in whole blood and in white cell-reduced red cells. *Transfusion* 2006; 46(4):589-94.
- Van der Meer PF, Eijzena M, Pietersz RNI. Comparison of two sterile connection devices and the effect of sterile connections on blood component quality. *Transfusion* 2006; 46(3):418-23.
- Van der Meer PF, Pietersz RNI. An evaluation of automated blood collection mixers. *Vox Sang* 2006; 91(3):275-7.

- Van der Schoot CE, Ait Soussan A, Koelewijn J, Bonsel G, Paget-Christiaens LG, de Haas M. Non-invasive antenatal RHD typing. *Transfus Clin Biol* 2006; 13(1-2):53-7.
- Van Hensbergen Y, Schipper LF, Brand A, Slot MC, Welling M, Nauta AJ, Fibbe WE. Ex vivo culture of human CD34+ cord blood cells with thrombopoietin (Tpo) accelerates platelet engraftment in a NOD/SCID mouse model. *Exp Hematol* 2006; 34(7):943-50.
- Van Manen HJ, van Bruggen RV, Roos D, Otto C. Single-cell optical imaging of the phagocyte NADPH oxidase. *Antioxid Redox Signal* 2006; 8(9-10):1509-22.
- Van Leeuwen EM, Koning JJ, Remmerswaal EB, van Baarle D, van Lier RA, ten Berge IJ. Differential usage of cellular niches by cytomegalovirus versus EBV – and influenza virus specific CD8+ T cells. *J Immunol* 2006; 177(8):4998-5005.
- Van Mirre E, Breunis WB, Geissler J, Hack CE, de Boer M, Roos D, Kuijpers TW. Neutrophil responsiveness to IgG, as determined by fixed ratios of mRNA levels for activating and inhibitory FcγRII (CD32), is stable over time and unaffected by cytokines. *Blood* 2006; 108(2):584-90.
- Van Raam BJ, Verhoeven AJ, Kuijpers TW. Mitochondria in neutrophil apoptosis. *Int J Hematol* 2006; 84(3):199-204.
- Van Thienen JV, Fledderus JO, Dekker RJ, Rohlena J, van Ijzendoorn GA, Kootstra NA, Pannekoek H, Horrevoets AJ. Shear stress sustains atheroprotective endothelial KLF2 expression more potently than statins through mRNA stabilization. *Cardiovasc Res* 2006; 72(2):231-40.
- Verhoeven AJ, Hilarius PM, Dekkers DW, Lagerberg JW, de Korte D. Prolonged storage of red blood cells affects aminophospholipid translocase activity. *Vox Sang* 2006; 91(3):244-51.
- Vidarsson G, Stemerding AM, Stapleton NM, Spliethoff SE, Janssen H, Rebers FE, de Haas M, van de Winkel JG. FcRn: an IgG receptor on phagocytes with a novel role in phagocytosis. *Blood* 2006; 108(10):3573-9.
- Voves C, Wuillemin WA, Zeerleder S. International Society on Thrombosis and Haemostasis score for overt disseminated intravascular coagulation predicts organ dysfunction and fatality in sepsis patients. *Blood Coagul Fibrinolysis* 2006; 17(6):445-51.
- Wagenaar-Bos IG, Hack CE. Structure and function of c1-inhibitor. *Immunol Allergy Clin North Am* 2006; 26(4):615-32.
- Wolbink GJ, Vis M, Lems W, Voskuyl AE, de Groot E, Nurmohamed MT, Stapel S, Tak PP, Aarden L, Dijkmans B. Development of antiinfliximab antibodies and relationship to clinical response in patients with rheumatoid arthritis. *Arthritis Rheum* 2006; 54(3):711-5.

Wouters D, Voskuyl AE, Molenaar ET, Dijkmans BA, Hack CE. Evaluation of classical complement pathway activation in rheumatoid arthritis: Measurement of C1q-C4 complexes as novel activation products. *Arthritis Rheum* 2006; 54(4):1143-50.

Zeerleder S, Schroeder V, Hack CE, Kohler HP, Willemin WA. TAFI and PAI-1 levels in human sepsis. *Thromb Res* 2006; 118(2):205-12.

Zeerleder S. The struggle to detect circulating DNA. *Crit Care* 2006; 10(3):142-4.

Zwaginga JJ, Nash G, King MR, Heemskerk JW, Frojmovic M, Hoylaerts MF, Sakariassen KS. Flow-based assays for global assessment of haemostasis. Part 1: biorheologic considerations. *J Thromb Haemost* 2006; 4:2486-7.

Zwaginga JJ, Sakariassen KS, Nash G, King MR, Heemskerk JW, Frojmovic M, Holyaerts MF. Flow-based assays for global assessment of haemostasis. Part 2: current methods and considerations for the future. *J Thromb Haemost* 2006; 4(12):2716-7.

Miscellaneous publications

Alphabetically by first author

Aalberse RC. Structural features of allergenic molecules. In: Cramer R, editor. *Allergy and Asthma in Modern Society: A Scientific Approach*. Basel: Karger, 2006: 134-146.

Beckers EA, Gonzalez-Garcia FN, Vrieling H. Herseninfarct door polycytemie als eerste uiting van een niercelcarcinoom [Brain infarction due to polycythemia as first indication of renal cell carcinoma]. *Ned Tijdschr Geneesk* 2006; 150(29):1642.

Curvers J, Groen P. Automatisering van de unit Productie binnen de bloedbanken. *NVB bulletin* maart 2006(1), 2-4.

Engelfriet CP, Reesink HW, Farrugia A, Schönitzer D, Wendel S, Olyntho S, Hansen MB, Kretschmer V, Karger R, Hassan HJ, Tadokoro K, Flanagan P, Solheim BG, Frey-Wettstein M, Caffrey E, Ruta M, Epstein JS. *International Forum*. Paid vs unpaid donors. *Vox Sang* 2006; 90:63-70.

Engelfriet CP, Reesink HW, Felfernig M, Sporn P, Kretschmer V, Karger R, Hansen E, Koopman-van Gemert AWMM, Thomas D, Thompson JF, Haynes S. *International Forum*. Perioperative blood salvage. *Vox Sang* 2006; 91:185-92.

Engelfriet CP, Reesink HW, Fontão-Wendel R, Lazar A, Cardoso RA, Olyntho S, Achkar R, Wendel S, Pisacka M, Taaning E, Koski T, Matilainen J, Kretschmer V, Karger R, Politis C, Katsea P, Malamou V, Aprili G, Piccoli P, Gandini G, Franchini M, Schonewille H, Brand A, Solheim BG, Flesland O, Seyfried H, Michalewska B, Letowska M, Tissot J-D, Milkins C, Knowles S,

DeSilva M, Contreras M, Stainsby D, Combs MR, Arney RS, Telen MJ. *International Forum*. Prevention and diagnosis of delayed haemolytic transfusion reactions. *Vox Sang* 2006; 91:353-68.

Engelfriet CP, Reesink HW, Henn G, Mayr WR, Olyntho S, Wendel S, Robillard P, Turek P, Krusius T, Koskinen S, Taaning E, Jørgensen , Rebibo D, Hauser L, Zorzi P, Legras J-F, Slimani A, Vo-Mai M, Trouvin J-H, andreu G, Kretschmer V, Keller-Stanislawski B, Mentzer D, Politis C, Lawlor E, Laspina S, Grazzini G, Hassan HJ, Aprili G, Okazaki H, Faber J-C, Benson S, Solheim BG, Letowska M, Selivanov E, Danilova T, Snopek I, Nel T, Martin MP, Senn M, Stainsby D, Cohen H, Jones H, Blumberg N, Braun MM, Malarkey MA, Epstein JS, Menitove JE, Lipton KS. *International Forum*. Haemovigilance. *Vox Sang* 2006; 90:207-41.

Engelfriet CP, Reesink HW, Körmöczy GF, Mayr WR, Panzer S, Schönitzer D, Fontão-Wendel R, Wendel S, Long A, Dziegiel MH, Kretschmer V, Karger R, Morelati F, Revelli N, Villa Ma, Lai M, de Haas M, van der Schoot E, Overbeeke M, Maaskant P, Dounder D, Flanagan P, Solheim BG, Zupanska B, Michalewska B, Muñoz-Diaz E, Martin-Vega C, Frey BM, Mendez A, Kochman Sa, Epstein JS, Garratty G, Lomas-Francis C, Westhoff CM. *International Forum*. Testing for weak D. *Vox Sang* 2006; 90:140-53.

Kerkhoffs JH. Te PAS en te onPAS: toepassing en Klinische effectiviteit van bloedplaatjes die worden bewaard in synthetische bewaarmedia. *Ned Tijdschr Hematol* 2006; 3:221-6.

Kerkhoffs JLH, Novotny VMJ. Klinische toepassing van synthetische bewaarvloeistoffen en fotochemische pathogeenreductie bij plaatjesconcentraten bij hemato-oncologische patiënten. *Ned Tijdschr Hematol* 2007; 4:23-5.

Lavenne-Pardonge E, Itegwaa MA, Kalaai M, Klinkenberg G, Loncke JL, Pelgrims K, Strengers PF. Emergency reversal of oral anticoagulation through PPSB-SD: the fastest procedure in Belgium. *Acta Anaesthesiol Belg* 2006; 57(2):121-5.

Overbeeke MAM. *Hematologie*. Arnhem: Syntax, 2006.

Pietersz RNI. Blood Components. In: *Global Perspectives in Transfusion Medicine* Eds. M. Lozano, M. Contreras, and M. Blajchmann. AABB 2006: pp 25-53.

Reesink HW. Hepatitis C virusremmers. *Topics in Hepatologie*. 2006;1, jaargang 4:3.

Schonewille H, Brand A. Prevention and diagnosis of delayed haemolytic transfusion reactions [International Forum]. *Vox Sang* 2006; 91(4):361-2.

So-Osman C, Nelissen RG. De Transfusie Op Maat studie-optimaal bloedmanagement binnen de orthopaedische chirurgie. *Ned Tijdschr Orthopedie* 2006; 13:159-62.

Strengers PF. Aanvallen van angio-oedeem bij kinderen door C1-esteraseremmerdeficiëntie [Episodes of angioedema in children with C1 esterase inhibitor deficiency]. *Ned Tijdschr Geneesk* 2006; 150(26):1480-1.

Van den Akker E, Oepkes D, Brand A, Kanhai HH. Vaginal delivery for fetuses at risk of alloimmune thrombocytopenia? *BJOG* 2006; 113(7):781-3.

Van Till JWO, Boormeester MA, Modderman PW, Sandick JW, Hart MH, Gisbertz SS, Lanschot JJ, Aarden LA. Variable Mannose-Binding Lectin Expression during Postoperative Acute-Phase Response. *Surg Infect* 2006; 7(5):443-52.

Van der Meer PF, Curvers J, Verhoeven AJ, Scharenberg J, Heeremans J, Kuipers W, Eijzenga M, De Cuyper IM, de Korte D, de Wildt-Eggen J. Nationale afstemming van het protocol om CD62P en annexine V binding in trombocytenconcentraten te meten. *NVB Bull* 2006; (5):37.

Late 2005

Beckers EA, Schonewille H, Kerkhoffs JL. Pathophysiology and treatment of sickle cell disease. *Ned Tijdschr Geneesk* 2005; 149:2024.

PhD theses

Martine Grootkerk-Tax

11 January 2006

Rh Variability in Multi-Ethnic Perspective – Consequences for RH Genotyping

Erasmus University Rotterdam

Promotor: Prof DJ van Rhenen MD PhD

Co-promotores: PA Maaskant-van Wijk PhD, CE van der Schoot MD PhD

Mariska Rondaij

16 January 2006

Regulation of Weibel-Palade body exocytosis in human endothelial cells

University of Utrecht

Promotor: Prof K Mertens PhD

Co-promotores: JJ Voorberg PhD, M Fernandez-Borja PhD

Brenda Luken

30 January 2006

Antibodies directed to ADAMTS13 in acquired thrombotic thrombocytopenic purpuras

University of Utrecht

Promotor: Prof K Mertens PhD

Co-promotores: JJ Voorberg PhD, R. Fijnheer PhD

Tamimount Mohammadi

8 February 2006

Bacterial contamination of platelet concentrates: Molecular tools and applications

Free University

Promotor: Prof CMJE Vandenbroucke-Grauls

Co-promotores: PHM Savelkoul MD PhD, HW Reesink MD PhD

Bahram Alamdary Badlou

13 March 2006

Prolonged platelet preservation by metabolic suppression

University of Utrecht

Promotor: Prof JWN Akkerman

Co-promotor: WM Smid MD PhD

Mettine Bos

28 March 2006

Regulatory mechanisms in the inactivation of blood coagulation factors V en VIII

University of Utrecht

Promotor: Prof K Mertens

Co-promotor: AB Meijer PhD

Marco Koppelman

28 June 2006

Viral safety of blood donations: molecular detection of blood transmittable viruses

University of Amsterdam

Promotor: Prof J Schuitemaker

Co-promotores: J Zaaijer MD PhD, HW Reesink MD PhD

Jean Paul ten Klooster

30 November 2006

Rac1 Targeting and Signalling - A hypervariable point of view

University of Amsterdam

Promotor: Prof D Roos

Co-promotor: PL Hordijk PhD

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