Scientific Report 2003

5 years of Sanquin

Contents

Introduction

5 years of Sanquin

Academic affiliations

5 years of Sanquin

Merger in 1998

In 1998 the Blood Banks and the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service merged into the Sanquin Blood Supply Foundation. Sanquin is actively trying to further strengthen its research efforts and is focusing its research program based on its mission to ensure the Dutch blood supply and to advance transfusion medicine in such a way that the highest possible standards are met in quality, safety and efficiency.

Starting from more then fifty years of research experience, Sanquin will increasingly endorse a corporate research program covering a broad range of areas within the framework of blood supply, transfusion medicine and all its threats and challenges. In this 2003 Scientific Report all research activities of Sanquin are covered, with the exception of contract research and plasma products development.

Historical developments

In the past, both within a number of blood banks and the Central Laboratory, basic and applied research was performed. Historically a number of research lines were started based on the then current threats to blood transfusion (i.e. HIV infections) and the need to investigate fundamental principles underlying the development of blood and plasma products as well as development of diagnostics.
The relatively small research departments of a number of blood banks collaborated closely with nearby university hospitals, as for example in Leiden. The much larger Central Laboratory (founded in 1943) succeeded in securing its own niche within the Dutch biomedical research landscape, and has since the 1980s a close collaboration with the Dept. of Clinical and Experimental Immunology of the University of Amsterdam. As a result of this niche position, basic, high quality, research was performed, next to – and often underpinning – applied developments.

What happened to the Sanquin research program in the five years of its existence?

The merger of 22 relatively small blood banks into nine – and later on four – regional blood bank divisions created the opportunity for larger and stronger research groups at those blood banks. For the former Central Laboratory the merger with the blood banks which produces mainly cellular products, implicated a stronger emphasis of its research program on cellular products. Decreasing funding possibilities from Sanquin's own resources, due to economic developments, strengthened the need for restructering of the research portfolio.

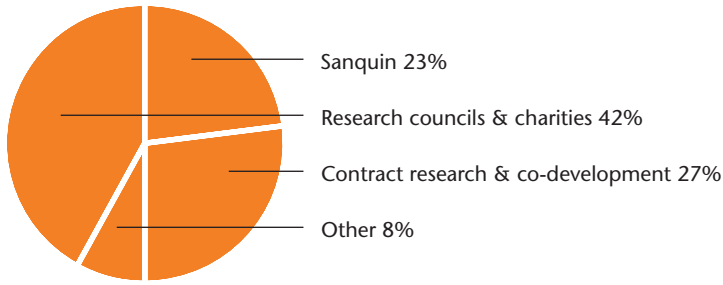
In the past five years much effort was put in strengthening corporate research. Based on Sanquin's mission (to provide the blood supplies and to promote transfusion medicine, in such a way as to meet the most stringent quality, safety and efficiency requirements) more research capacity was put in blood bank research departments. Basic research lines and research projects further a field from Sanquin core business were being trimmed down or even discontinued.

Was the niche position of the Central Laboratory in the past decisive for its position in The Netherlands, Sanquin now relies more and more on collaboration with university research departments, both within the Netherlands and abroad, for basic research. However, Sanquin finds it extremely important to be able to keep performing basic research, albeit not curiosity driven but mission oriented. Without knowledge and practical experience in basic research, evidence based transfusion medicine and development of safe and efficient blood and plasma products and therapies will not be possible.

In our research policy – as laid down annually in the five year research planning document – a focus towards more transfusion medicine related research has led to a growth in research on immunohematology, blood cell biology, stem cell research, pathogen inactivation, new cellular therapies and investments in donor related studies,

epidemiology and cost effectiveness studies. There is an increasing interest in clinical studies in collaboration with hematologists and oncologists in academic and general hospitals at both blood bank divisons and Sanquin Research. Recently, research into alternatives for human blood derived products has again been put on the agenda. The research lines on HIV, allergy and cellular immunology are being trimmed down.

Sources of funding of research projects (direct costs only)



Professionalizing research management

In the past five years, co-ordination of research on central level was strengthened by the installation of the Research Programming Committee. This Committee – consisting of the Directors of the divisions Research, Diagnostic Services and Plasma Products and one of four directors of Sanquin blood banks divisions – advises Sanquin's Executive Board on start and continuation of research projects, maintaining infrastructure and purchasing of expensive research equipement. Recently, also proposals for external funding need approval of the Programming Committee.

On each of the stakes plasma products, diagnostic services and cellular products, working groups of senior investigators decide on the proposals to be written, in close collaboration between all divisions involved. Active researchers discuss progress of research projects and new ideas in project groups in order to generate new proposals for funding from both internal and external sources.

Sanquin finds it extremely important to be able to keep performing basic research, albeit not curiosity driven but mission oriented.

Quality assessment: *ex ante* and *ex post*

Sanquin assumes that all research, both basic and applied, within its divisions should be of high quality. Research funded from external sources usually is assessed *ex ante* in competition by peer review.
Research funded by Sanquin it self is being assessed (also in competition) by the Research Programming Committee and its working groups. Current research projects are evaluated annually.

A recurrent site visit system by peer review committees was already introduced a number of years ago. The core of the peer review committees are formed by members of the Research Assessment Board, consisting of Dutch as well as international members. All research groups will be peer reviewed once in every five years. In 2003 no site visits took place.

In order to be able to maintain high quality research, human resource management aiming at a balanced academic staff profile and an accompanying management development program were put in place.

The Scientific Advisory Board supervises the quality system in place and checks annualy whether Sanquin's research program meets the framework of the five year planning document.

Scientific Advisory Board

Prof EJ Ruitenberg PhD, Chairman (Utrecht University & Vrije Universiteit Amsterdam)
Prof A Brand MD PhD (Sanquin Blood Bank South West Region & Leiden University)
Prof HC Hemker MD PhD (Maastricht University)
A de Jonge PhD (Semaia Pharmaceuticals; untill August 2003)
Prof MM Levi MD PhD (University of Amsterdam)
Prof JWM van der Meer MD PhD (University of Nijmegen)
Prof DKF Meijer PhD (University of Groningen)
Prof F Miedema PhD (Sanquin Research)
Prof DJ van Rhenen MD PhD (Sanquin Blood Bank South West Region & Erasmus University Rotterdam)
Vacancy as from August 2003
JW Smeenk MSc, Executive secretary (Sanquin Corporate Staff)

Research Programming Committee

Prof F Miedema PhD, Chairman (Sanquin Research)
C Aaij PhD (Sanquin Diagnostic Services)
Prof DJ van Rhenen MD PhD (Sanquin Blood Bank)
RF Tiebout MD (Sanquin Plasma Products)
JW Smeenk MSc, Executive secretary (Sanquin Corporate Staff)

Research Assessment Board

National members

Prof RM Bertina MD PhD (Leiden University)
Prof FC Breedveld MD PhD (Leiden University)
Prof WE Fibbe PhD (Leiden University)
Prof F Grosveld PhD (Erasmus University Rotterdam)
Prof ADME Osterhaus PhD (Erasmus University Rotterdam)
Prof JJ Sixma MD PhD (Utrecht University)

International members

Prof D Anstee MD, PhD (University of Bristol, United Kingdom)
Prof R Carell PhD (University of Cambridge, Cambridge, United Kingdom)
Prof RA Flavell PhD (Yale University, New Haven, USA)
Prof LW Hoyer MD (American Red Cross, Rockville, MD, USA)
Prof MD Kazatchkine MD PhD (INSERM, Hospital Broussais, Paris, France)
Prof RA Koup MD PhD (University of Texas, Southwestern Medical Center, Dallas, USA)
Prof D Lane MD PhD (Imperial College School of Medicine, London, United Kingdom)

Scientific publications

Year	*total number*	*SCI publications*	*Theses*	*Average impact factor*
2003	187	144	6	4.59
2002	177	142	7	4.70
2001	173	131	12	4.06
2000	169	137	12	4.64
1999	204	166	9	4.03
1998	222	167	7	4.41
1997	264	197	14	3.95
1996	281	204	11	4.07
1995	272	192	16	3.89
1994	230	185	11	na
1993	226	179	12	na

Articles* published in 1993 through 1998 annual reports cited** in five full years after publication

Publications from	*Total citations*	*Number of SCI publiccations*	*Citations in year*					
			1998	1999	2000	2001	2002	2003
1993	3483	179	615					
1994	3599	185	686	552				
1995	3215	192	732	641	622			
1996	3057	204	736	685	615	530		
1997	2962	197	369	661	657	656	619	
1998	3448	167		646	811	768	646	577

* *Only SCI publications are included*

** *Excluding self citations*

Academic affiliations

Education & training in blood transfusion medicine, immunology, and laboratory techniques

research@sanquin.nl
www.practicaltraining.sanquin.nl
www.scs.sanquin.nl

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. Sanquin staff members participate in research programmes and curricula of the AMC Research Institute for Immunology (JJ van Loghem Institute) and the Research Institute for Infectious Diseases. The joint AMC – Sanquin Landsteiner Laboratory is housed within Sanquin premises.

At many Dutch universities, staff from various Sanquin divisions are involved in theoretical and practical training programmes for undergraduate and graduate students in (medical) biology, pharmacy, medicine, and health sciences as well as laboratory technicians. Of course, Sanquin is also involved in training of specialist in blood transfusion medicine, other medical specialties, and training of nurses.

Sanquin Consulting Services emphasizes on training on the job for colleagues from sister organizations in devloping countries in Africa, South America, and Asia as well as the former East European Countries. Recently a Masters program was established in collaboration with the University of Groningen.

CAF-DCF established two chairs at the Catholic University of Leuven:
Prof dr Michel Delforge, CAF-DCF professor in Hematology and Stem Cell Plasticity, and Prof dr Jacques Pirenne, CAF-DCF professor in Abdominal Transplant Surgery

Staff from various Sanquin divisions are involved in theoretical and practical training programmes for undergraduate and graduate students.

Professorships Sanquin Staff

Prof RC Aalberse PhD (Biological immunology, Faculty of Biology, University of Amsterdam)
Prof LA Aarden PhD (Molecular immunology, Academic Medical Center, University of Amsterdam)
Prof A Brand MD PhD (Blood transfusion medicine, Faculty of Medicine, Leiden University)
Prof CE Hack MD PhD (Immunopathophysiology of non specific immunity, Free University, Amsterdam)
Prof TW Kuijpers MD PhD (Pediatric immunology, Emma Children's Hospital, University of Amsterdam)
Prof K Mertens PhD (Pharmaceutical plasma proteins, Faculty of Pharmacy, Utrecht University)
Prof F Miedema PhD (Immunology of AIDS, Academic Medical Center, University of Amsterdam)
Prof DJ van Rhenen MD PhD (Blood transfusion medicine, Faculty of Medicine and Health Sciences, Erasmus University Rotterdam)
Prof D Roos PhD (Non-specific immunology, Academic Medical Center, University of Amsterdam)
Prof CTh Smit Sibinga MD PhD (International development of transfusion medicine, University of Groningen)

‘Blood transfusion needs to be a scientifically based practice’

‘…critical peer review is essential to guarantee a scientific dialogue…’

It is commonsensical that we have merged into one blood provision organization. Both quality and safety need to be addressed from one point of view. Products need to be exchangeable within the whole of Europe. It is much easier to talk to all parties involved as a single national organization, especially where legislative and regulatory measures from the EC are becoming more and more stringent.

I see the task of the Scientific Advisory Board as safeguarding the high scientific level by assessing scientific quality. Our view is that transfusion medicine needs a scientific foundation. A transfusion organization lacking that scientific base does not know when it is working with out of date principles. In an academic environment, critical peer review is essential to guarantee a scientific dialogue. With that, Sanquin ensures support in the scientific community both within The Netherlands and worldwide.

The Advisory Board in its present form was created in 2001. At its start an inventory of research in the area of blood transfusion was done. We feel that research at blood bank divisions in collaboration with academic medical centers is necessary. In that way Sanquin uses and attributes to the knowledge infrastructure.

One of the Advisory Board’s main tasks is writing the yearly updated five year planning document on research, in consultation with the Medical Advisory Board. Also we assess quality of research in retrospect, looking at the reports of site visits by international peer review committees. Finally we explore research at academic centers and look for missing issues and advise Sanquin’s Executive Board for instance on creating personal professorships.

‘…research in collaboration with academic medical centers is necessary…’

Interview with Professor E. Joost Ruitenberg, Chairman Scientific Advisory Board

Research lines

Hematology

Hemostasis and thrombosis

Inflammation and sepsis

Immunology

Blood transmitted infections

Quality, safety and efficiency

New therapies and evaluation of clinical applications

Donor studies, epidemiology and cost effectiveness

Hematology

Allo-immunity against blood group antigens

Principal investigators
Ellen van der Schoot MD
PhD Dept Experimental Immunohematology
(e.vanderschoot@sanquin.nl)
Masja de Haas MD
PhD Dept Experimental Immunohematology
(m.dehaas@sanquin.nl)

In the last year the projects dealing with the molecular characterization of the Rhesus system have been finished. Especially, variation in the Rh-locus in Negroids was studied.

A non-invasive diagnostic method for RhD typing on fetal DNA circulating in maternal plasma, based on detection of *RHD* sequences, was tested on more than 2500 D-neg pregnant women. This study shows the feasibility of screening D-negative women to restrict antenatal immunoprophylaxis to women carrying D-positive fetuses. In coming years our research will focus on the development of therapeutic approaches. For this purpose, a cross-reactive anti-idiotype against anti-RhD antibodies is being developed by means of phage technology. The role of IgG-Fc receptors (the activating receptors FcγRIIa,- IIIa and -IIIb as well as the inhibiting FcγRIIb) polymorphisms in blood cell destruction has been further investigated.

As part of a cost-effectiveness analysis of the new Dutch policy on screening for irregular red cell antibodies and antenatal RhD immunoprophylaxis, we continued with a case-control trial including two-year cohorts of pregnant women with a positive screening test for clinically relevant irregular red cell antibodies. This is a co-operative study together with prof Bonsel from the University of Amsterdam. Furthermore, a study was started to elucidate whether the antenatal RhD immunoprophylaxis has led to a reduction in RhD immunizations. The outcome of these studies may lead to changes in the screening policy. Research was done in collaboration with Dr. Petra Maaskant-van Wijk (Blood Bank South West Region, Rotterdam), Dr Lieve Christiaens (University Medical Center, Utrecht), and Dr. Arend Mulder (Leiden University Medical Center).

Key publications

Engelfriet CP, Reesink HW, Judd WJ, Ulander VM, Kuosmanen M, Koskinen S, Rouger P, Morelati F, Tantalo V, Fujii T, de Haas M, van der Schoot CE, Overbeeke M, Koelewijn J, Bonsel G, Vrijkotte T, Zupanska B, Martin-Vega C, Parra Lopez R, de Silva M, Contreras M, Panzer S, Ulm B, Mayr WR. Current status of immunoprophylaxis with anti-D immunoglobin. Vox Sang. 2003; 85(4):328-37.

As part of a cost-effectiveness analysis a case-control trial was set up for clinically relevant irregular red cell antibodies, including two-year cohorts of pregnant women.

Hemker MB, Cheroutre G, van Zwieten R, Maaskant-van Wijk PA, Roos D, Loos JA, van der Schoot CE, von dem Borne AE. The Rh complex exports ammonium from human red blood cells. Br J Haematol. 2003; 122(2):333-40.

Van der Schoot CE, Tax GH, Rijnders RJ, de Haas M, Christiaens GC. Prenatal typing of Rh and Kell blood group system antigens: the edge of a watershed.Transfus Med Rev. 2003; 17(1):31-44.

Molecular blood group studies

Principal investigator
Petra Maaskant-van Wijk PhD Dept Research and Education, South West Region (petra.maaskant@bloodrtd.nl)

The aim is to evaluate the application of molecular blood group typing of red cell and platelet antigens for blood bank purpose and for diagnostic and therapeutic purposes, in particular for patients with a non-Caucasian origin or for transfused patients with (multiple) antibodies. More fundamental research relates to understanding of the relationship between expression of blood groups and organisation at the molecular level.

Research of the molecular background of weak D expression confirmed that weak D expression is not caused by decreased amounts of *RHD* mRNA transcripts. In the erythroleukemic cell-line model, K562, transfection of weak RhD type 1 and 3 lead to decreased expression of RhD with comparable amounts of *RHD* mRNA transcripts as found in cells expressing normal RhD. This indicates that the T809G (weak D type 1) and the C8G (weak D type 3) mutations cause weak D expression at a post transcriptional level. Scatchard plot analysis and flow cytometry confirmed lower epitope densities but did not indicate qualitative alteration of RhD in weak D expression.
A proposed mechanism of *RHD* deletion has been described. It is now possible to determine *RHD* zygosity with a PCR-RFLP assay. This is applicable in case of RhD-allo-immunized RhD-negative pregnant women to determine whether the father is homozygous for *RHD*, leading always to an affected child, or heterozygous, when there is a 50% chance that the child is not affected. The assay is currently tested for reliability in patients with different ethnic backgrounds. In Caucasians, the most

We contribute to a European project that aims to validate with different ethnic populations a micro-array designed for typing of *ABO*, *RHD* and a set of clinically relevant red cell antigens.

common cause of RhD negativity is *RHD* deletion. In non-Caucasians three forms of RhD negativity exist; r's gene (*RHD*(1-3)-RHCE(3-7)-*RHD*(8-10) hybrid gene), *RHD* pseudogene and the *RHD* deletion. A real time quantitative PCR assay on fetal DNA isolated from maternal plasma was developed to predict fetal RhD status with these forms of RhD negativity taken into account. This assay is now validated on blood samples from RhD negative pregnant women from Curacao (high frequency of *RHD* pseudogene). In collaboration with the Blood Bank Shangdong from China the cause of RhD negativity in Chinese Han population is being investigated.

For various other blood groups genotyping by pyrosequencing for the detection of SNPs (Single Nucleotide Polymorphisms), which are the molecular basis of most blood groups has been used. The pyrosequencing technique is a 'middle-throughput' technique suitable for a blood bank setting. Assays to genotype for KEL, JK, GATA FY and HPA-1, -2, -3 and -5 have been developed. We established a panel of HPA-1, and -5 donors and identified 18 HPA-1a negative donors who are currently on call for plateletpheresis for cases of neonatal allo-immune thrombocytopenia. A four year Sanquin program started in 2003 to compare serology with genotyping using the pyrosequencing technique on a large scale. For the development and implementation of a real 'high-throughput' system, the transfusion medicine DNA micro-array, there is collaboration with Sanquin Research/Diagnostic Services, the Blood Bank North West Region and JT den Dunnen (Human and Clinical Genetics, Leiden). A European Consortium, of which the Blood Bank South West Region is a subcontractor, has been formed to demonstrate the use of molecular genetic techniques to genotype a large cohort of individuals drawn from across the EU in order to demonstrate the accuracy and improvement of this technology over standard serological testing.

Key publications

Hemker MB, Cheroutre G, van Zwieten R, Maaskant-van Wijk PA, Roos D, Loos JA, van der Schoot CE, von dem Borne AE. The Rh complex exports ammonium from human red blood cells. Br J Haematol. 2003; 122(2):333-40.

Tax MG, van der Schoot CE, van Doorn R, Douglas-Berger L, van Rhenen DJ, Maaskant-vanWijk PA. RHC and RHc genotyping in different ethnic groups. Transfusion. 2002; 42(5):634-44.

Granulocyte activation

NADPH oxidase

Principal investigators
Prof Dirk Roos PhD
Dept Experimental Immunohematology
(d.roos@sanquin.nl)
Prof Taco W Kuijpers MD PhD Dept Experimental Immunohematology and Emma Childrens Hospital AMC
(t.w.kuijpers@amc.uva.nl)

Phagocytic leukocytes generate reactive oxygen species as a defence against pathogenic micro-organisms. The enzyme responsible for this reaction is an NADPH oxidase. In resting, non-phagocytosing cells, the various subunits of this enzyme are located in different compartments, with the actual enzymatic subunit, a b-type flavocytochrome, in the membranes of secretory vesicles and specific granules, and a complex of activity-regulating proteins in the cytosol. Upon neutrophil activation, these subunits come together in the plasma membrane or in the phagosomal membrane surrounding the phagocytosed pathogen. The enzyme is activated by complex formation, allowing access of the substrate NADPH to the catalytic center and donation of electrons to free oxygen. This activation process is controled by the small GTPase Rac2 and may also be influenced by Rap1A. We have studied this activation process by expressing GFP-tagged Rac2 or GFP-tagged p67-phox (one of the cytosolic oxidase components) in a myeloid cell line. The tagged proteins translocated to the plasma membrane during phagocytosis, dependent on the presence of the flavocytochrome. However, we found with fluorescence recovery after photobleaching (FRAP) that a continuous interaction of Rac2 and p67-phox exists with the flavocytochrome: the oxidase complex is in dynamic equilibrium with its components. In future, we will study the interaction of all three of these components at the same time in living cells by fluorescence resonance energy transfer (FRET) (in collaboration with Dr C Otto, Technical University Twente). We have now also expressed GFP-tagged Rap1A wild-type and mutants in this cell line to study the involvement of this GTPase in the activation or de-activation of the NADPH oxidase. In addition, we have constructed cell-permeable peptides that may interfere with Rap1A function; preliminary experiments indicate that these peptides do interfere with cell movement but not with oxidase activity.
We have extensively studied priming of human neutrophils with various TLR ligands. Furthermore, we have started to unravel the signal transduction pathways used by different TLRs to prime the respiratory burst. We found that the same signal

transduction routes are used by soluble stimuli for TLRs as well as particles that are taken up and stimulate TLRs. We were also able to show that TLRs signal both from the cell surface as well as from intracellular compartments.

Opsonization

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 are investigating the importance of mannose-binding lectin (MBL) in the opsonization of zymosan (yeast particles) and various bacteria species. We found that MBL is absolutely required for zymosan opsonization, but in its absence, anti-zymosan antibodies can partially take over the opsonization function. In case of Staphylococcus, both MBL and Ig-mediated complement activation are necessary for proper opsonization. We are now investigating the relation between MBL level in plasma (measured with a functional assay of MBL binding to immobilized mannan) and opsonizing capacity. For this purpose we use plasma from 10 donors with a heterozygous MBL exon 1 codon 52 mutation but with different mannan-binding capacity. We will measure deposition of MBL, C4 and C3 on zymosan, promoter polymorphisms of MBL, and degree of MBL oligomerisation. In addition, we are developing a high-throughput method to detect the relevant mutations and polymorphisms in the MBL gene. The clinical relevance of MBL deficiency is studied in several pediatric patient groups, among them patients with juvenile rheumatoid arthritis, neonatal patients with fever, follow-up patients cured from solid tumours or leukemia/lymphoma's, acne patients and oncology patients under chemotherapy and/or irradiation. Moreover, a start was made with a phase-II clinical trial with MBL suppletion in pediatric oncology patients under similar treatment.
We also found that amplification of complement activation by the positive feedback loop of the alternative pathway is essential for adequate opsonization of zymosan. We obtained evidence that mutations in MBL predispose patients with Kawasaki Disease to coronary complications. In collaboration with Japanese colleagues we have also started to isolate and study the properties of ficolin/p35 species, lectins with a similar function as MBL.

Key publication

van Bruggen R, Anthony E, Fernandez Borja M and Roos D. Continuous translocation of Rac2 and the NADPH oxidase component p67^{phox} during phagocytosis. J. Biol. Chem. 2004; 279:9097-102.

Apoptosis

Apoptosis of neutrophils is an important mechanism of regulating the duration of an inflammatory response. Several cytokines are known to affect this process, e.g. G-CSF, GM-CSF and interferon-γ (IFN-γ) prolong neutrophil survival, whereas TNFα enhances neutrophil apoptosis. In the past year, we have investigated in more detail the role of the mitochondria in the induction of apoptosis in neutrophils. These organelles appear to be different in neutrophils from other cell types, because they preserve mainly death-mediating abilities and hardly participate in the cellular metabolism. Upon induction of apoptosis with TNFα and cycloheximide, the neutrophil mitochondria release a number of pro-apoptotic proteins into the cytosol, which facilitate activation of the caspase cascade. Intact activation of the initiator caspase-9 in the virtual absence of cytochrome c in neutrophils suggests that in these cells the intrinsic pathway of apoptosis may operate in a different way than in other cell types. Nevertheless, during apoptosis, the neutrophil mitochondria release into the cytosol massive amounts of pro-apoptotic factors, such as Smac/DIABLO and Omi/HtrA2. G-CSF blocks the translocation of Bid and Bax (pro-apoptotic Bcl-2 homologues) to the mitochondria as well as truncation of Bid, thus preventing subsequent mitochondrial leakage and activation of caspase-8, -9 and -3, which accompany spontaneous apoptosis. These data support an integrative role of the mitochondria in induction and/or amplification of caspase activity. Furthermore, we have investigated the cause of neutropenia in two groups of patients, one with glycogen storage disease type 1b (GSD1b) and another with Barth Syndrome (BTHS). The circulating neutrophils of both groups bind Annexin V, indicating the possibility of preliminary apoptosis. Indeed, GSD1b neutrophils display several additional apoptotic features, such as increased caspase activity, condensed nuclei and perinuclear clustering of mitochondria to which Bax had translocated. In contrast, BTHS neutrophils did not show any other apoptotic phenotype and were also not phagocytozed by macrophages, thus excluding early clearance as an explanation for the neutropenia.

We do not know the cause of neutropenia in BTHS, nor do we understand the increased Annexin V binding by BTHS neutrophils, but we conclude that apoptosis should never be judged exclusively by Annexin V binding.

Key publications

Maianski NA, Roos D and Kuijpers TW. Tumor necrosis factor-α induces a caspase-independent death pathway in human neutrophils. Blood 2004; 101:1987-95.

Kuijpers TW, Maianski NA, Tool ATJ, Smit GPA, Rake JP, Roos D and Visser G. Apoptotic neutrophils in the circulation of patients with glygogen storage disease 1b (GSD1b). Blood 2004; 101:5021-4.

Signaling in transendothelial migration

Principal investigator
Peter Hordijk PhD
Dept Experimental Immunohematology
(p.hordijk@sanquin.nl)

Research in this group is following two research lines: (i) GTPase mediated signaling in leukocyte migration, and (ii) adhesion-induced signaling in endothelial cells regulating cadherin-mediated cell-cell adhesion and leukocyte transendothelial migration. These lines of research are complementary since many signaling components are involved in both cell types, resulting in a well integrated and complementary series of research projects.

A new model for primary human stem cells

The study on a new model for primary human stem cells was completed in 2003. This model was developed by retrovirally expressing a CXCR4-GFP fusion protein in the CXCR4-deficient human hematopoietic progenitor cell line KG1a. This KG1a CXCR4-GFP cell line showed full restoration of SDF-1 responsiveness in assays detecting activation of ERK1/2 phosphorylation, actin polymerization, adhesion to endothelium under conditions of physiological flow, and (transendothelial) chemotaxis. When adhered to cytokine-activated endothelium in the absence of SDF-1, CXCR4 did not localize to the leading edge of the cell but was uniformly distributed over the plasma membrane. In contrast, when SDF-1 was immobilized on cytokine-activated endothelium, the CXCR4-GFP receptors that were present on the cell surface markedly

Data indicate that VCAM-1-induced, Rac-dependent signaling plays a key role in the modulation of vascular-endothelial cadherin-mediated endothelial cell-cell adhesion and leukocyte extravasation

redistributed to the leading edge of migrating cells. In addition, CXCR4-GFP co-localized with lipid rafts in the leading edge of SDF-1-stimulated cells. Inhibition of lipid raft formation prevents SDF-1-dependent migration, internalization of CXCR4, and polarization to the leading edge of CXCR4, indicating that CXCR4 surface expression and signaling requires lipid rafts.

SDF-1 signaling in leukocyte migration

Major effectors of SDF-1 signaling in leukocyte migration are the Rho-like GTPases. We have developed a line of research on the signaling specificity of these GTPases, based on detailed analysis of protein-protein interactions mediated by their hyper-variable C-termini. Using cell-permeable versions of these C-terminal domains, we found that the C-termini of Rac1, Rac2, RhoA, and Cdc42, interfere with GTPase signaling in a specific fashion in a variety of cellular models. Moreover, the C-terminus of Rac1 (but not Rac2 or Cdc42) binds to phosphatidylinositol 4,5-phosphate kinase (PIP5K) and associates to the adapter protein Crk via the N-terminal Src homology 3 (SH3) domain of Crk and the proline-rich stretch in the Rac1 C-terminus. These interactions mediate Rac1 localization, as well as Rac1 signaling toward membrane ruffling, cell-cell adhesion, and migration. These data show that the C-terminal, hypervariable domain of Rac1 encodes two distinct binding motifs for signaling proteins and regulates intracellular targeting and differential signaling in a unique and non-redundant fashion.

Adhesion-molecule-induced signaling in the endothelial cells

In addition, we found that the p42 and p38 MAP kinases contribute importantly to leukocyte chemotaxis, and studies are ongoing to establish the molecular details of the role of these kinases in receptor turnover, actin polymerization and cell polarization and motility.

On their way out of the circulation, leukocytes cross the endothelial monolayer, lining the blood vessels. This extravasation is associated with, and potentiated by adhesion-molecule-induced signaling in the endothelial cells. We found that antibody-mediated cross-linking of VCAM-1 on IL-1beta-activated primary human umbilical vein endothelial cells induced actin stress fiber formation, contractility, and formation of intercellular gaps. The effects induced by VCAM-1 cross-linking were inhibited by C3 toxin,

implicating involvement of the small GTPase p21Rho. In addition, the effects of VCAM-1 were accompanied by activation of Rac, which we recently showed to induce intercellular gaps in pHUVEC in a Rho-dependent fashion. With the use of a cell-permeable peptide inhibitor, it was shown that Rac signaling is required for VCAM-1-mediated loss of cell-cell adhesion. Furthermore, VCAM-1-mediated signaling toward cell-cell junctions was accompanied by, and dependent on, Rac-mediated production of reactive oxygen species (ROS) and activation of p38 MAPK. In addition, it was found that inhibition of Rac-mediated signaling blocks transendothelial migration of monocytic U937 cells. Together, these data indicate that VCAM-1-induced, Rac-dependent signaling plays a key role in the modulation of vascular-endothelial cadherin-mediated endothelial cell-cell adhesion and leukocyte extravasation.

This line of research is currently being continued by studies on the endothelial docking structure, which is formed upon adhesion of leukocytes by the apical membrane of the endothelial cells and which appears to concentrate the signaling machinery that is required for efficient adhesion and transendothelial migration. Additional, ongoing studies deal with the control of cadherin function in endothelial cells by microtubules and associated proteins, using a combination of microinjection, live cell confocal microscopy and real time monitoring of endothelial electrical resistance. Similarly, we have extended our studies on the proteins that generate endothelial ROS and are particularly focusing on the NOX4 protein, its localization and regulation in endothelial cells. Finally, a new research line was initiated focusing on the role of Robo and Slit proteins in interaction between hematopoietic progenitor cells and endothelium. This work will be expanded significantly in 2004.

Key publications

Van Buul JD, Voermans C, van Gelderen J, Anthony EC, van der Schoot CE, Hordijk PL. Leukocyte-endothelium interaction promotes SDF-1-dependent polarization of CXCR4. J Biol Chem. 2003; 278: 30302-10.

Van Hennik PB, ten Klooster JP, Halstead JR, Voermans C, Anthony EC, Divecha N, Hordijk PL. The C-terminal domain of Rac1 contains two motifs that control targeting and signaling specificity. J Biol Chem. 2003; 278:39166-75.

Van Wetering S, van den Berk N, van Buul JD, Mul FP, Lommerse I, Mous R, ten Klooster JP, Zwaginga JJ, Hordijk PL. VCAM-1-mediated Rac signaling controls endothelial cell-cell contacts and leukocyte transmigration. Am J Physiol Cell Physiol. 2003; 285:C343-52.

Red cell research

DNA arrays

Principal investigators
Prof Dirk Roos PhD
Dept Experimental Hematology
(d.roos@sanquin.nl)
Rob van Zwieten ing
Dept Experimental Hematology
(r.vanzwieten@sanquin.nl)
Masja de Haas MD PhD Dept Experimental Hematology and Sanquin Diagnostic Services
(m.dehaas@sanquin.nl)

Two years ago, we have started a project in collaboration with investigators from the Center of Human Genetics of the University of Leiden to detect a large number of hemoglobin mutations by DNA array techniques. Several methods of mutation detection were tested, such as single nucleotide primer extension, allele-specific oligonucleotide amplification and oligonucleotide ligase amplification. In all cases, the oligonucleotides were carrying a tag, for detection of the labeled product by arrays spotted with probes hybridising to the tags. Both glass arrays and three-dimensional flow-through arrays (PamArrays from PamGene, Den Bosch, The Netherlands) were tested. The flow-through arrays have the advantage that different hybridisation conditions can be used to achieve optimal hybridisation. To type blood donors for clinically relevant red cell blood group and platelet antigen systems by microarray, a multiplex PCR amplifying 19 gene fragments was developed. In a pilot study with glass arrays and probe sets designed for human platelet antigen typing, it was shown with two different panels of typed donors (n=94) that correct typing was achieved in all cases. The micro-array will now be extended with probes to type for the blood group antigen systems of red cells. Furthermore, we started to contribute to a European project that aims to validate with different ethnic populations a micro-array designed for typing of *ABO*, *RHD* and a set of clinically relevant red cell antigens.

Key publication

Van Moorsel CH, van Wijngaarden EE, Fokkema IF, den Dunnen JT, Roos D, van Zwieten R, Giordano PC, Harteveld CL. ß-Globin mutation detection by tagged single-base extension and hybridization to universal glass and flow-through microarrays. Eur J Hum Genet 2004; 12:567-73.

Phospholipid asymmetry in red cells

Principal investigator
Arthur J Verhoeven PhD
Dept Transfusion Technology
(a.verhoeven@sanquin.nl)

Previous studies on the effect of photodynamic treatment (PDT) for the inactivation of pathogens in red cell concentrates (RCC) have indicated loss of phospholipid asymmetry of the plasma membrane due to activation of phospholipid scrambling. We now have investigated maintenance of phospholipid asymmetry during long-term storage of RCC at 4°C. Loss of phospholipid asymmetry in the membrane of erythrocytes results in exposure of phoshatidylserine (PS) and to rapid removal from the circulation.

Two activities affecting phospholipid asymmetry were investigated: the ATP-dependent translocase (or flippase, transporting PS from outer to inner leaflet) and scrambling activity. Flippase was measured by determining the inward translocation of NBD-PS by flow cytometry, scrambling activity was measured by determining the inward translocation of NBD-phosphatidylcholine. PS exposure was measured with AnnexinV-FITC.

The results of this study show that, in contrast to many other blood cell types, phospholipid scrambling is absent in red cells, both directly after isolation and after storage at 4°C for 7 weeks. This results in good maintenance of phospholipid asymmetry during storage at 4°C, as indicated by the absence of cells able to bind Annexin V (<5%). We did observe, however, a decrease in flippase activity starting after 4 weeks of storage, which reached 50% of the control value after 7 weeks. This decrease strongly correlated with the decrease in cellular ATP levels, but rejuvenation of RBC after 7 weeks to increase ATP levels only partially restored flippase activity. Possibly, a decrease in total enzyme activity also plays a role in this phenomenon.

A decrease in flippase activity does compromize the red cells in their ability to shuttle phosphatidylserine from the outer leaflet of the plasma membrane back to the inner leaflet. Indeed, when fresh and stored RCC were exposed to the same conditions of PDT, the percentage AnnexinV-positive cells was significantly higher in RCC stored for 7 weeks as compared to fresh RCC. It is not known yet whether the decrease in flippase activity is of relevance for the *in vivo* performance of red cells after transfusion.

Key publication

Hilarius PM, Ebbing IG, Dekkers DW, Lagerberg JW, de Korte D, Verhoeven AJ. Generation of singlet oxygen induces phospholipid scrambling in human erythrocytes. Biochemistry 2004; 43(13):4012-9.

Oxygen delivery by human RBC

Principal investigators
Arthur J Verhoeven PhD
Dept Transfusion Technology
(a.verhoeven@sanquin.nl)
Dirk de Korte PhD Dept
Transfusion Technology
(d.dekorte@sanquin.nl)

To allow pre-clinical testing of experimental RCC, a collaborative project with the department of Physiology of the Amsterdam Medical Center to determine red cell function *in vivo*, was started in 2001. Studies were focused on a rat model, in which hemodilution was carried out to an hematocrit of 15%, after which part of the rat blood cells (about 30%) was replaced by human red cells. Human RCC were washed several times to remove residual plasma, containing crossreacting antibodies against rat antigens. Microvascular oxygen tension in the gut was measured to determine local oxygen delivery.

In this rat model, a clear effect of storage of the RCC was observed as freshly isloated human red cells were able to maintain oxygen tension, whereas RCC stored for 5-6 weeks did not. Surprisingly, standard RCC stored for 2-3 weeks (in which 2,3-DPG had disappeared), were also able to support oxygen delivery, indicating that another factor than 2,3-DPG might be responsible for the storage-induced defect in oxygen delivery. Further studies into the underlying mechanism of this deficiency are ongoing.

Red cell aging and survival: Improvement of blood processing and storage conditions on aging and *in vivo* survival of red blood cells

Principal investigator
Joyce Curvers PhD Dept
Research and Education
South East Region
(j.curvers@sanquin.nl)

The measurements of cell-bound IgG on stored red blood cells (under various conditions) by using an enzyme-linked antiglobulin test have been ended. Red blood cells were measured weekly during storage. No significant storage-related change of the quantity of cell-bound IgG and no significant storage-related change of the binding capacity for autologous IgG (after recombination with autologous plasma) could be seen. The presence of apparently low-avidity cell-bound IgG was detected and its clinical relevance will be investigated by means of a phagocytosis assay.

For the measurement of phagocytosis of stored red blood cells we have chosen to use a flowcytometric method. Therefore we have isolated and frozen a pool of buffy coat-derived monocytes. We are currently optimising this assay and we will follow the *in vitro* sensitivity of red blood cells with different storage times to phagocytosis. These results will be used to study the relationship with the *in vivo* survival of transfused red blood cells. In order to measure this *in vivo* survival we have previously developed a sensitive flowcytometric method and we are currently designing a protocol to measure this survival in patients.

The method for measuring ATP (by bioluminescence) was adopted and can be used for a longitudinal study of stored red blood cells.
In the upcoming year we will initiate a longitudinal study following stored red blood cells. During this study we will measure metabolic and physical changes, binding of IgG and phagocytosis.
This research is performed in collaboration with the Dept of Biochemistry, NCMLS; the University Medical Center, Nijmegen; the Dept of Transfusion Services, University Medical Center, Nijmegen and Rijnstate Hospital, Arnhem. Executive staff: M Luten (junior researcher), Sanquin Blood Bank South East Region; B Roerdinkholder-Stoelwinder (research technician), Sanquin Blood Bank South East Region; Dr HJ Bos (project coordinator), Sanquin Blood Bank South East Region; Dr GJCGM Bosman, Dept. of Biochemistry, University Medical Center, Nijmegen; Prof WJ de Grip, Dept of Biochemistry, University Medical Center, Nijmegen; Dr JM Werre, Dept of Transfusion Services, University Medical Center, Nijmegen and FLA Willekens, Rijnstate Hospital, Arnhem.

Key publication

Luten M, Roerdinkholder-Stoelwinder B, Bos HJ, Bosman GJCGM – Cell-bound IgG as an ageing related parameter for the quality of stored red blood cell. Blood 2003; 102(11):4251 (abstract).

Hemostasis and thrombosis

Biosynthesis of the factor VIII-von Willebrand factor complex

Principal investigators
Jan A van Mourik PhD
Dept Plasma Proteins
(j.vanmourik@sanquin.nl)
Jan J Voorberg PhD
Dept Plasma Proteins
(j.voorberg@sanquin.nl)

While factor VIII and von Willebrand factor (VWF) circulate in plasma in a non-covalent complex, it has remained controversial whether or not cells exist that are capable of expressing the combination of both proteins. It has been generally accepted that the liver is a major site of factor VIII synthesis. Employing a murine model, we have previously demonstrated that factor VIII mRNA synthesis occurs both in the liver and in non-hepatic tissues such as kidney and brain. Hepatic factor VIII synthesis is primarily confined to cells lining the sinusoids. These cells, however, are devoid of VWF mRNA. The lack of co-localisation of factor VIII and VWF synthesis in these and other liver cells supports the view that complex assembly occurs after the constituent proteins have entered the circulation. This issue was further explored in hepatic tissue of patients with severe liver disease. Because liver failure often is associated with elevated factor VIII levels in plasma, the possibility was addressed that this would be associated with increased expression of hepatic factor VIII mRNA. This, however, could not be confirmed by quantitative gene expression analysis. However, we did find enhanced hepatic synthesis of VWF mRNA, as well as increased plasma levels of VWF. By virtue of its stabilising effect on factor VIII, VWF may cause the elevated factor VIII levels in liver disease. We further found reduced levels of mRNA encoding low density lipoprotein receptor-related protein (LRP). This suggests that impaired LRP-dependent clearance of factor VIII (see below) may also contribute to elevated factor VIII in severe liver failure. Whether or not this factor VIII is from extra-hepatic origin, remains an open question for further study.

As for VWF, it was established that it is synthesized in vascular endothelial cells, where it is stored in typical organelles, the Weibel-Palade bodies. Besides VWF, these Weibel-Palade bodies contain other proteins, including P-selectin and the chemotactic cytokine interleukin-8 (IL-8). Upon stimulation by agonists such as thrombin or epinephrin, Weibel-Palade bodies undergo exocytosis, resulting in release or surface expression of their contents. Studies on the trafficking and secretory behavior of a hybrid of VWF with green fluorescent protein (VWF-GFP) have demonstrated that

It seems well conceivable that small GTP-ase plays a key role in the trafficking of Weibel-Palade bodies and the regulated secretion of VWF from endothelial cells.

VWF plays an active role in sequestering IL-8 into these endothelial storage organelles. Real-time confocal laser scanning microscopy revealed a surprising complexity of Weibel-Palade body dynamics, including perinuclear redistribution of a subset of Weibel-Palade bodies that apparently escape secretion. An interesting question is which intracellular pathways drive the trafficking of Weibel-Palade body subsets in endothelial cells. One potential mechanism involves the small GTP-binding protein Ral. We have previously shown that activation of endothelial cells by thrombin results in transient cycling of Ral from its inactive GDP-bound to its active GTP-bound state. In other cell types, Ral seems to be involved in cytoskeleton dynamics and rearrangement. Therefore, it seems well conceivable that this small GTP-ase indeed plays a key role in the trafficking of Weibel-Palade bodies and in the regulated secretion of VWF from endothelial cells. The significance of Ral and a variety of other effector molecules in the trafficking of VWF-GFP containing Weibel-Palade bodies is currently under investigation.

Key publications

Hollestelle MJ, Thinnes T, Crain K, Stiko A, Kruijt JK, Van Berkel TJ, Loskutoff DJ, Van Mourik JA. Tissue distribution of factor VIII gene expression *in vivo* – a closer look. Thromb. Haemostas. 2001; 86:855-61.

Van Mourik JA, Romani de Wit T, Voorberg J. Biogenesis and exocytosis of Weibel-Palade bodies (review). Histochem Cell Biol 2002; 117:113-22.

Structure and function of enzyme-cofactor complexes

Principal investigator
Prof Koen Mertens PhD
Dept Plasma Proteins
(k.mertens@sanquin.nl)

One characteristic of the coagulation cascade is that it comprises several serine proteases that act in combination with a non-enzymatic cofactor. We focus on the complex of activated factor IX (factor IXa) and its cofactor factor VIII, and in particular on the relation between cofactor binding and enhancement of factor X activation by factor IXa. By protein engineering studies of the factor IXa protease domain (the heavy chain), we previously demonstrated that multiple surface-exposed loops in the protease domain limit the accessiblity of the substrate binding groove. This makes enzyme activity strictly dependent on the presence of its cofactor. Upon factor VIII

binding, intramolecular rearrangements facilitate substrate binding and cleavage by a so far unrecognized mechanism. Our studies have demonstrated that factor VIII binding involves two helical structures in the factor IXa protease domain (residues 301-303 and 333-339). In addition to these factor VIII-interactive sites in the factor IXa heavy chain, also the factor IXa light chain contributes to assembly with factor VIII. We have observed that in particular the interface between the two Epidermal Growth Factor-like (EGF) domains in the factor IXa light chain contribute to the factor VIII-dependent rate enhancement. Recombinant factor IX chimeras that contained an elongated EGF-linking segment displayed reduced response to both the complete activated factor VIII molecule and to its isolated A2 domain. This is a surprising observation, because the same mutants still displayed normal association with the factor VIII A2 domain in surface plasmon resonance studies. Because the A2 domain is thought to interact with the helix regions in the factor IXa heavy chain, it seems likely that the EGF-linking region has an allosteric effect on factor IXa catalytic activity. We are currently trying to unravel the mechanism of intramolecular signaling between the light chain and the catalytic site in the factor IX protease domain.

Key publications

Mertens K, Celie PHN, Kolkman JA, Lenting PJ. Factor VIII-factor IX interactions: molecular sites involved in enzyme-cofactor complex assembly (review) Thromb. Haemostas. 1999; 82:209-17.

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.

Inhibitory antibodies in hemophilia

Principal investigator
Jan J Voorberg PhD
Dept Plasma Proteins
(j.voorberg@sanquin.nl)

Coagulation factor replacement therapy of hemophilia may be complicated by the formation of inhibitory or neutralising antibodies (inhibitors). This side-effect occurs in approximately 25% of the patients with severe hemophilia A, and in about 5% in patients with severe hemophilia B. Since a few years we have been analysing the anti-factor VIII antibody repertoire of a variety of patients with acquired and congenital

factor VIII deficiency employing phage display technology. Individual IgG4 antibodies are obtained as single-chain variable domain antibody fragments (scFv), and these fragments then are subjected to sequence analysis and detailed epitope mapping analysis employing recombinant factor VIII fragments. By this approach we have been able to identify antibodies against epitopes in the factor VIII domains A2, A3 and C2. As for the C2 domain, more than 80% of the inhibitor patients have antibodies against this domain in their circulation. Epitope mapping of these antibodies have demonstrated the presence of a predominant inhibitor epitope comprising residues 2248-2313. Antibodies against the C2 domain prevent factor VIII from binding to phospholipid membranes to its carrier protein VWF. This notion is compatible with the presence of binding sites for lipids and for VWF in the C2 domain. Although the inhibitory mechanism and epitope specificity of C2 inhibitors are well understood, knowledge on the primary structure of these antibodies has remained limited.
We have previously found that the variable heavy chain (VH) regions of anti-C2 antibodies are encoded by the closely related germ line segments DP-10, DP-14 and DP-88, which all belong to the VH1 gene family. We further identified additional anti-C2 antibodies that are derived from the VH gene segments DP-88 and DP-5. Competition studies with murine monoclonal antibodies demonstrated that DP-5 and DP-88 derived antibodies bind to distinct epitopes on the C2 domain. Epitope mapping studies employing factor VIII/factor V hybrids revealed that carboxy-terminal residues 2223-2332 are required for binding of the DP-10, DP-14 and DP-88 encoded antibodies. In contrast, binding of the DP-5 encoded antibodies required residues in both the amino- and carboxy-terminus of the C2 domain. Moreover, replacement of residues 2181-2243 of human factor VIII by those of porcine factor VIII did not affect binding of the DP-10, DP-14 and DP-88 derived antibodies, but abrogated binding of the DP-5 encoded antibodies. We therefore conclude that these two groups of antibodies target two distinct sites in the factor VIII C2 domain.

Key publications

Van den Brink EN, Turenhout EAM, Bovenschen N, Heijnen BG, Mertens K, Peters M, Voorberg J. Multiple VH genes are used to assemble human antibodies directed toward the A3-C1 domains of factor VIII. Blood 2001; 97:966-72.

Van den Brink EN, bril WS, Turenhout EAM, Zuurveld M, Bovenschen N, Peters M, Yee TT, Mertens K, Lewis DA, Ortel TL, Lollar P, Scandella D, 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.

Cellular receptors involved in clearance of factor VIII and factor IX

Principal investigator
Prof Koen Mertens PhD
Dept Plasma Proteins
(k.mertens@sanquin.nl)

The mechanism of clearance of coagulation factors VIII and IX has long remained poorly understood. A few years ago, we and others observed that factor VIII binds to the low-density lipoprotein receptor-related protein (LRP), a receptor that is involved in the binding and cellular uptake of a variety of ligands. We further observed that LRP binds to factor IXa, but not to its non-activated precursor factor IX. This suggests that LRP interacts with structure elements that become surface-exposed upon factor IX activation, possibly the same sites that also are involved in factor IXa function. We have employed a large panel of factor IX variants with substitutions in various surface loops in the protease domain in order to map the LRP binding site. This approach revealed that LRP binding involves residues Phe-342 to Asn-346, which are located in a surface loop at the entrance of the substrate binding cleft of the factor IXa serine protease domain. The LRP binding site probably is more extended, and may comprise basis amino acids that are surrounding residue Asn-346. A number of potentially important residues has been identified, and the role thereof is currently analyzed in more detail. One particularly striking observation is that LRP binding inhibits factor IXa activity towards its natural substrate factor X. This suggests that LRP, apart from its role in the cellular uptake of factor IXa, contributes to the regulation of factor IXa within the coagulation cascade.

As for factor VIII, others have reported that LRP binding is limited to the A2 domain in the factor VIII heavy chain. In our laboratory we consistently find that the isolated factor VIII light chain displays the same high-affinity for LRP binding as the intact factor VIII heterodimer of heavy and light chain. By using a combination of synthetic

This suggests that LRP, apart from its role in the cellular uptake of factor IXa, contributes to the regulation of factor IXa within the coagulation cascade.

peptides, recombinant antibody fragments, and factor VIII/factor V hybrid molecules, we have demonstrated that LRP binding involves the residues 1811-1818 in the A3 domain. This suggests that the LRP binding site is partially overlapping with a factor IXa interactive region, which is known to involve the same surface-exposed part of the factor VIII A3 domain. We propose that multiple factor VIII light chain regions contribute to LRP binding, some of which may represent interactive sites involved in assembly with factor IXa.

We have further addressed the question whether or not the interaction of LRP with factor VIII has any physiological implication. For this purpose we have used a mouse model of conditional hepatic LRP deficiency. This model takes advantage of the so-called 'cre-lox-P' technique for targeted disruption of the LRP gene. Upon inactivation of the LRP gene, mice developed significantly higher factor VIII plasma levels than their non-deficient controls, and these persisted for at least 6 weeks. LRP deficient mice further displayed longer factor VIII half-life in infusion studies using purified human factor VIII. These findings provide the first direct evidence that the endocytic receptor LRP indeed regulates factor VIII clearance *in vivo*. Adenovirus-mediated over-expression of the endocytic receptor antagonist Receptor Associated Protein (RAP) also resulted in an increase of factor VIII levels. Surprisingly, this occurred both in normal mice and in mice that were lacking hepatic LRP. This suggests that apart from LRP, also other RAP-sensitive receptors contribute to the regulation of factor VIII levels in the circulation. The identification of these receptors is a major challenge, which may have significant impact on factor VIII pathophysiology. Dysfunction of LRP or its related receptors may cause elevated factor VIII levels and as such be a novel risk factor for developing venous thrombosis.

Key publication

Bovenschen N, Boertjes RC, van Stempvoort G, Voorberg J, Lenting PJ, Meijer AB, Mertens K. Low density lipoprotein receptor-related protein and factor IXa share structural requirements for binding to the A3 domain of coagulation factor IXa. J. Biol. Chem. 2003; 278:9370-7.

Inflammation and sepsis

Immunoglobulins

Principal investigator

Prof C Erik Hack Dept of Immunopathology (e.hack@sanquin.nl)

Aim of these studies is to get better insight into the working priciples of IvIG in different diseases, and to get further information about the physico-chemical properties of immunoglobulins in general and of IvIG in particular.

Biological properties of intravenous immunoglobulin

Research on the biological properties of IvIG has centered on the interaction with Fc-receptors. In cooperation with the Dept Experimental Immunohematology, specific PCRs were set up to measure expression of the different Fcγ-Receptors II (FcγRII) on cells, which include the activating FcγRIIa and the inhibitory FcγRIIb. The ratio between these activating and inhibitory receptors was determined on various cell types. On neutrophils this ratio was found to correlate with elastase release upon triggering with IgG-dimers. This technology will be used to screen patients that react with and without side effects upon IvIG infusion. In an individual patient with side effect, an abnormal FcγRIIa was found, which is further characterized.

Biochemical and structural aspects of intravenous immunoglobulin preparations

Research on the biochemical and structural aspects of immunoglobulins and IvIG has been dedicated to the proteolytic breakdown of IgG in IvIG, on the presence of pepsin, and on the aggregation process of IgG molecules. Proteases involved in the degradation of IvIG were identified. In addition, experiments were started to determine the structure of IgG by setting up an expression system for IgG. Currently, the influence of the hinge region on structure and stability is being investigated.

Key publication

Van Mirre E, Teeling JL, van der Meer JW, Bleeker WK, Hack CE. Monomeric IgG in intravenous Ig preparations is a functional antagonist of FcgammaRII and FcgammaRIIIb. J Immunol 2004; 173(1):332-9.

Auto-immune Diseases

Principal investigator
Prof Lucien Aarden, Dept of Immunopathology (l.aarden@sanquin.nl)

The Auto-immune Diseases Research 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 eryhtematosus (SLE). An assay for clusterin, a main protein binding to apoptotic cells, was developed, concentrations of this protein in various diseases have been measured. As model for antibody formation in auto-immune conditions, antibody formation to Infliximab in patients with rheumatoid arthritis (RA) was investigated. Infliximab (anti-TNF monoclonal antibody) treatment is nowadays a standard treatment of RA, but clinical responses become limited over time in most patients. In cooperation with the department of Rheumatology of the VU Medical Center (Dr GJ Wolbink a.o.) studies levels of Infliximab and anti-Infliximab antibodies were measured in RA patients. Formation of Infliximab-neutralizing antibodies indeed seems to be major cause for diminished clinical efficacy. This research is now extended to other anti-TNF agents.
In the Auto-immune Diseases group work on immunosuppressive and anti-inflammatory drugs in the whole blood system as an *ex vivo* model, also was continued. Part of this work was done in cooperation with pharmaceutical companies. This has led to a clinical trial of a compound in patients with RA. The results of this trial are being evaluated. Finally, studies on the role of Toll-like receptors in the whole blood system were continued.

Key publications

De Lathouder S, Gerards AH, de Groot ER, Valkhof MG, Dijkmans BA, Aarden LA. Bioassay for detection of methotrexate in serum. Scand J Rheumatol 2004; 33(3):167-73.

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. Auto-immunity 2004; 37(2):95-102.

Results in patients with rheumatoid arthritis (RA) indicated that levels of these activation parameters may provide some clues about activity of the disease.

Inflammation

Principal investigator
Prof C Erik Hack, Dept of Immunopathology (e.hack@sanquin.nl)

The Inflammation Research centers around identification of novel activation products of the classical pathway of complement and on the development and clinical evaluation of complement inhibitors. A major inhibitor of complement is C1-inhibitor, a serpin. Identification of the function of some other serpins, in particular in apoptosis is also a topic of this research-line. Previously it was found that covalent fixation of activated C4 and C3 to C1q occurred during classical pathway activation and not during other activation processes. A differential antibody sandwich ELISA was optimized, and levels of these novel activation products were measured in various diseases. Results in patients with rheumatoid arthritis (RA) indicated that levels of these activation parameters may provide some clues about activity of the disease. The work on the structure and function of the classical pathway inhibitor C1-inhibitor was continued. Based on previous results of a study of a genetic deficiency of C1-Inhibitor a novel recombinant mutant of C1-inhibitor was designed, as were mutants that lack any carbohydrate group. These mutants are currently expressed in *Pichia pastoris* and will be analysed for pharmacokinetics and dynamics *in vivo* in rabbits. A novel project on the role and specificity of IgM in ischemia-reperfusion was started together with the Dept of Experimental Surgery of the AMC, and the immunoglobulin group of the Dept of Immunopathology.

Key publications

Bos IG, Lubbers YT, Roem D, Abrahams JP, Hack CE, Eldering E. The functional integrity of the serpin domain of C1-inhibitor depends on the unique N-terminal domain, as revealed by a pathological mutant. J Biol Chem 2003; 278(32):29463-70.

Strik MC, Wolbink A, Wouters D, Bladergroen BA, Verlaan AR, van Houdt IS, Hijlkema S, Hack CE, Kummer JA. Intracellular serpin SERPINB6 (PI6) is abundantly expressed by human mast cells and forms complexes with beta-tryptase monomers. Blood 2004; 103(7):2710-7.

Immunology

Immunopathology of HIV infection

Chronic immune activation is the major driving force for CD4+ depletion

Principal investigator
Prof F Miedema PhD Dept Clinical Viro-Immunology (f.miedema@sanquin.nl)

A long standing line of research is directed towards the pathogenic mechanism by which HIV-1 infection causes depletion of CD4+ T-cells, immune deficiency and ultimately AIDS. In the past year, we have shown that increased peripheral cell division rates that characterize HIV-1 infection are caused by chronic immune activation through the persistently active virus, rather then by a homeostatic response to T-cell depletion. HIV-1 infection is characterized by chronic generalized CD8 and CD4 T-cell hyperactivation, the biological impact of which is not completely understood. We have proposed recently that chronic immune activation may in fact be the driving force for depletion of in particular the naive CD4 T-cell pools, which are the most difficult to replenish and will hence lead to CD4+ T-cell depletion.
HIV negative Ethiopians have lower CD4+ T-cell counts compared to any other population in Africa or in industrialized countries. We studied whether this unique immunological profile results in a shorter survival time upon HIV infection in 149 factory workers, participating in an open cohort study in Ethiopia. Using a Markov-model, we estimated a median survival time in the range of 9.1 to 13.0 years, which is very similar to that of populations in industrialized countries before the advent of antiretroviral therapy. HIV-infected Ethiopians turned out to have a significantly lower annual CD4 T-cell loss compared to 185 Dutch HIV+ drug users and 308 HIV+ homosexual men who were followed in the Amsterdam cohort studies. Even when groups with similar CD4 T-cell counts were compared, the Ethiopians lost their CD4 T-cells at a significantly slower pace. This difference in CD4 T-cell loss was not merely due to lower level of HIV-1 RNA load or absence of syncytium-inducing/X4 HIV-type C viruses in Ethiopians.

This finding seems difficult to reconcile with the hyper immune activation hypothesis that was developed based on data from European and US cohorts. One would expect more rapid disease progression in Ethiopian HIV-patients. Even if Ethiopian and Dutch individuals experience the same antigenic challenge during HIV infection it is surprising that they have similar survival times since Ethiopians start off with

significantly lower CD4 T-cell counts. It has been shown that with aging there is gradual erosion of the naive T-cell pool which is likely the effect of exposure to foreign antigens and decreasing renewal. To survive longer with frequent infectious diseases, one has to have an efficient immune response but should at the same time minimize the accelerated aging of the immune system by curtailing bystander responses. It may be that compared to people in the Western world, Ethiopians and people living in the developing world in general have been strongly selected for the ability to survive despite chronic immune activation due to the high burden of infectious diseases.

We continued that line of work in studies on HIV infection in children. Current understanding of how the T-cell pool is established in children, and how this is affected by HIV infection, is limited. It is widely believed that the thymus is the main source for T-cells during childhood. Healthy children, however, showed an age-related increase in total body numbers of naive and memory T-cells while absolute numbers of T-cell receptor excision circles did not increase. This suggests that the establishment of the T-cell pool after birth is more dependent on T-cell proliferation than was previously recognized. Indeed, the proportion of dividing T-cells was high, especially in younger children, which is in good agreement with expansion through proliferation. In untreated HIV-1 infected children, total body numbers of T-cells and TRECs were low and stable, while T-cell division levels were highly increased. Using a mathematical model, we found that reduction of thymic output may not affect T-cell numbers to the extent that increasing the level of immune activation does. Our data therefore indicate that in HIV-infected children, similar to HIV-infected adults, continuous activation of the immune system may be a major factor lowering CD4 T-cell numbers.

CD70 Tg mice: A mouse model for chronic immune activation

In CD70 Tg mice the constitutive expression of CD70, the ligand of the costimulatory molecule CD27, results in a state of persistent immune activation. In these mice a number of phenomena are found that are considered to be key immunological features of HIV-1 induced immunodeficiency: evidence for increased T-cell turnover, diminution of the naive T-cell compartment and a progressive inability to respond *ex vivo* to antigen and mitogenic stimuli. To get a better insight in the consequences

of persistent T-cell activation the dynamics of the T-cell compartment are now studied in depth in these mice. These studies involve heavy water (D_2O) and BrdU labelling studies to estimate turnover rates of naïve and effector/memory T-cells and thymectomy and administration of IL-7 to analyse the contribution of thymic output. Ultimately the results of these studies will be compared to results in HIV patients.

Quantitative and qualitative aspects of virus-specific cellular immunity

Despite readily detectable virus specific $CD8^+$ T-cells in most HIV-infected patients, immune surveillance is eventually lost, leading to progression to AIDS. In addition, loss of control over Epstein-Barr Virus (EBV), a widespread human γ herpesvirus, may cause AIDS-related Non Hodgkin's Lymphomas (AIDS-NHL). Furthermore, Cytomegalovirus (CMV) infection can cause serious clinical complications in HIV-infected subjects. This line of research aims to unravel the role of cellular immunity against different viruses in HIV-infection.

Phenotypic and functional analysis of virus-specific T-cells in the course of HIV-infection

A detailed longitudinal analysis was performed to investigate HIV-specific $CD4^+$ T-cell responses during acute infection in individuals. Although $CD4^+$ T-cell function is generally lost in HIV infection, it has been reported that early HAART during acute HIV-1 infection may rescue HIV-1 specific $CD4^+$ T-cell responses which could protect from subsequent disease progression when the patient stops therapy. We performed a longitudinal analysis in 12 treated (of whom 6 subsequently underwent therapy interruption) and 6 non-treated individuals with a well-documented acute HIV-1 infection. Peripheral blood mononuclear cells (PBMC) were stimulated with overlapping peptide pools derived from gag and nef. Production of IFNγ and IL-2 by $CD4^+$ T-cells was analyzed in combination with proliferative responses.
Absolute numbers of gag-specific $CD4^+$ T-cells producing IL-2, IFNγ, or both were higher in treated (n=6) compared to untreated individuals (n=6) up to 2 years after seroconversion. HAART during acute HIV-1 infection was associated with lower viral load and preserved proliferative capacity of HIV and non-HIV-specific $CD4^+$ T-cells. Two out of six individuals that discontinued therapy showed viral control. Three had

Our data show that IL-2 and IFNγ producing capacity of HIV-specific CD4+ T-cells is spared by early HAART and can be expanded in some patients by increasing viral load.

persistently increased viral load, paralleled by strong proliferative HIV-specific CD4+ T-cell responses and preserved numbers of cytokine producing CD4+ T-cells. Our data show that IL-2 and IFNγ producing capacity of HIV-specific CD4+ T-cells is spared by early HAART and can be strongly expanded in a fraction of patients by increasing viral load during treatment interruption.

EBV-specific CD8+ T-cells have been extensively studied in both healthy and HIV-infected individuals. In contrast, few data are available on EBV-specific CD4+ T-cell responses. To study these responses, PBMC were stimulated with overlapping peptide pools from 2 EBV proteins. After direct *ex vivo* stimulation, latent antigen EBNA1 or lytic antigen BZLF1-specific IFNγ-producing CD4+ T-cell numbers were low (0.03 to 0.36% of CD4+ T-cells), and measurable in less than half of the subjects (either HIV- and HIV+). Therefore, an assay was developed which allowed specific and reproducible antigen-specific expansion of EBV-specific CD4+ T-cells, independent of HLA type and *ex vivo* antigen processing. Untreated HIV-infected individuals had a lower CD4+ T-cell response to both EBNA1 and BZLF1 as compared to healthy EBV carriers and HAART-treated HIV+ subjects. This suggests loss of EBV-specific CD4+ T-cells due to HIV infection, while HAART might restore this response. Interestingly, numbers of EBV-specific CD4+ T-cells inversely correlated with EBV viral load, suggesting an important role for EBV-specific CD4+ T-cells in the control of EBV *in vivo*.

Dynamics of CMV-specific T-cells in HIV-1-infected individuals progressing to AIDS with CMV end-organ disease

Since CMV infection can cause serious clinical complications in immunocompromized individuals, we assessed cellular immune requirements for protection against CMV end-organ disease in HIV-1 infection. To this end longitudinal samples from HIV-1-infected patients in the Amsterdam cohort were analyzed, including progressors to AIDS with or without CMV end-organ disease and long-term non-progressors in the absence of HIV and CMV treatment.

Dynamics of CMV-specific CD8+ T-cell responses were analyzed by four-color fluorescence analysis using MHC class I CMV-peptide tetramers, and intracellular staining for perforin and granzyme B as well as IFNγ-production after stimulation

Increasing numbers of CMV infected cells in the face of high numbers of CMV-specific perforin$^+$granzyme B$^+$ CD8$^+$ T-cells may explain immune pathology characteristic for CMV disease.

with CMV-specific peptide. CMV-specific CD4$^+$ T-cells were measured as IFNγ-producing T-cells after stimulation with CMV-lysate. In parallel, CMV load was measured.
In progressors to AIDS with CMV end-organ disease, CMV-specific IFNγ$^+$CD4$^+$ T-cells disappeared in the year before onset of CMV end-organ disease, whereas these cells could still be detected in long-term non-progressors. This loss in CMV-specific CD4$^+$ T-cells was accompanied by a sharp increase in CMV load prior to onset of CMV-disease. CMV load could not be detected in either long-term non-progressors and progressors to AIDS without CMV-diesease. Despite increasing numbers of CMV-specific CD8$^+$ T-cells in both long-term non-progressors and progressors to CMV-disease, decreasing numbers of CMV-specific IFNγ-producing CD8$^+$ T-cells were found over time in patients progressing to CMV-disease. In contrast, the fraction of CMV-specific CD8$^+$ T-cells that contained perforin and granzyme B increased.
Our data indicate that insufficient help of CMV-specific CD4$^+$ T-cells may cause loss of CMV-specific IFNγ-CD8$^+$ T-cells and loss of control of CMV dissemination. Increasing numbers of CMV infected cells in the face of high numbers of CMV-specific perforin$^+$granzyme B$^+$ CD8$^+$ T-cells may explain immune pathology characteristic for CMV disease.

Development of HLA class II tetramers

To develop reagents that can be used to directly visualize antigen-specific CD4$^+$ T-cells, we have developed an expression system for HLA class II molecules fused to a peptide epitope sequence in insect cells. For several HLA class II molecules (HLA-DR1, HLA-DR3 and HLA-DR4) we have demonstrated production of the relevant molecules by the cells in tissue culture supernatant. These molecules can be readily purified and after biotinylation, tetramers can be formed. Recently HLA-DR3 molecules complexed with a Mycobacterium tuberculosis Ag85 or a CMV pp65 derived peptide have been shown to detect antigen-specific T-cell responses *ex vivo* in peripheral blood samples of exposed individuals. Specificity of the binding has been proven by an increase in the percentage of positive cells after antigen specific driven proliferation. Studies using the CMV specific HLA class II tetramer to determine the number and phenotype of the CMV specific CD4$^+$ cells in different patient populations are being set.

We demonstated that multiple and independent aberrations in the MHC class II antigen processing pathway are strongly related to disease progression.

Key publications

Hazenberg MD, Otto SA, van Benthem BHB, Roos MTL, Coutinho RA, Lange JMA, Hamann D, Prins M, Miedema F. Persistent immune activation in HIV-1 infection is associated with progression to AIDS. AIDS 2003; 17(13):1881-8.

Hazenberg MD, Borghans JA, de Boer RJ, Miedema F. Thymic output: a bad TREC record. Nat Immunol 2003; 4(2):97-9. Review.

Van Baarle D, Kostense S, van Oers MH, Hamann D, Miedema F. Failing immune control as a result of impaired CD8+ T-cell maturation: CD27 might provide a clue. Trends Immunol 2002; 23(12):586-91.

Antigen Presentation

Principal investigator
Marieke van Ham PhD
Dept of Immunopathology
(m.vanham@sanquin.nl)

Antigen Presentation Research was started last year as a separate research line to address the question how antigen presentation influences normal and abnormal antibody responses. Two types of cells will be studied for this, i.e. B-cells and dendritic cells.

This research line is a close collaboration with the Immunoglobulin Research group of the department and the Department of Autoimmunity of Sanquin Diagnostic Services. The research line was started by continuation of the work, that dr van Ham initiated at the Netherlands Cancer Institute (NKI) and later the VU Medical Center (VUmc), on the regulation and cellular mechanisms that lead to an efficient class II MHC molecules in normal and malignant B-cells. The project is done in cooperation with the departments of Hematology and Pathology of VUmc. 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 regulating the composition of the peptide repertoire. A series of malignant B-cells of patients suffering from B-cell chronic lymphocytic leukemia abnormal expression of these proteins were found. Moreover, we demonstated that multiple and independent aberrations in the MHC class II antigen processing pathway are strongly related to disease progression. Currently the clinical and functional implications of these findings are investigated. Together with

the department of Experimental Immunohematology this research line was expanded by starting a project on culturing dendritic cells for application in clinical protocols. Methods to analyse the functional state of these cells are developed currently. 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 initiated by continuation of a tetramer project, that originated at the Department of Viro-Immunology.

Allergy

Principal investigators
Prof Rob Aalberse PhD
Dept Immunopathology
(r.aalberse@sanquin.nl)
Ronald van Ree PhD
Dept Immunopathology
(r.vanree@sanquin.nl)

The Allergy Research group is focused on the formation of IgE antibodies against allergens. To have better screeningsassays work also has been done on the production of pure (recombinant) allergens. At the end this work should help to reduce clinical conditions like rhinitis, asthma as well as food allergy.
Most of the work on inhalant allergens is carried out in collaboration with allergen manufacturers. At present, diagnosis and allergen-specific immunotherapy are performed with almost crude allergen extracts. Hence, the work on cloning and expression of recombinant inhalant allergens has been continued. The availability of such allergens should facilitate studies on the specificity of IgE antibodies against these compounds.
Food allergy research is mainly performed in the frame of two EU-funded projects under the fifth framework program. These projects focus at the characterization of allergens in fruits and nuts. Work on the characterization of these allergens and antibody responses against them was continued.

Key publication

Akkerdaas JH, Wensing M, Knulst AC, Krebitz M, Breiteneder H, de Vries S, Penninks AH, Aalberse RC, Hefle SL, van Ree R. How accurate and safe is the diagnosis of hazelnut allergy by means of commercial skin prick test reagents?. Int Arch Allergy Immunol 2003; 132(2):132-40.

Blood transmitted infections

Virological aspects of AIDS pathogenesis

Chemokine receptors and the biological phenotype of HIV-1

Principal investigator
Hanneke Schuitemaker
PhD Dept Clinical Viro-Immunology
(h.schuitemaker@sanquin.nl)

HIV-1 infection is established by virus variants that use coreceptor CCR5 (R5 variants) in addition to the principal receptor CD4. In the course of infection, CXCR4-utilizing (X4) variants emerge but only in approximately 50% of infected patients. Factors that determine evolution towards X4 HIV-1 have not been resolved yet. We studied the role of $CCR5^+$ and $CXCR4^+$ target cell availability in virus phenotype evolution and disease progression at a time point before and 1 and 5 years after seroconversion in a prospective study of 102 individuals with a documented moment of HIV-1 seroconversion. High percentages of $CCR5^+$ cells among total cells (relative hazard [RH], 2.55; 95% confidence interval [95% CI], 0.99-6.52, but not among CD45RO-$CD4^+$ and $CD45RO^+$ $CD4^+$ cells preseroconversion and among total cells and CD45RO- $CD4^+$ cells (RH, 2.70; 95% CI, 1.06-6.92 and RH, 3.54; 95% CI, 1.27-9.90, respectively) 5 years after seroconversion were associated with more rapid progression to AIDS. One year after seroconversion, high percentages of $CXCR4^+$ cells among total and CD45RO- $CD4^+$ cells were associated with delayed development of X4 variants (RH, 0.49; 95% CI, 0.20-1.21 and RH, 0.41; 95% CI, 0.17-1.02, respectively), whereas no association was observed for the percentage of $CCR5^+$ cells. In a larger study population, high early serum viral RNA and low $CD4^+$ T-cell numbers were associated with more rapid development of X4 variants. Thus, our results exclude target cell availability as a driving force for R5-to-X4 virus phenotype evolution.

X4 variants evolve from R5 variants and after the first appearance of X4 variants they coexist with R5 variants. We have previously observed an ongoing genetic evolution with a continuous divergence of envelope gp120 sequences of coexisting R5 and X4 virus variants over time. This suggested the existence of two separate target cell niches for R5 and X4 variants. However, when we studied evolution of gag p17 sequences in two patients who developed X4 variants in the course of infection, we observed that the gag p17 sequences of R5 and X4 virus populations intermingled in phylogenetic trees and did not diverge from each other over time. Statistical evaluation using the Shimodaira-Hasegawa test indicated that the different genomic regions evolved along

different topologies, supporting the hypothesis of recombination. Our data imply that recombination between R5 and X4 HIV-1 variants occurs *in vivo* and implicates that cells expressing both CCR5 and CXCR4 can be infected simultaneously with an R5 and X4 variant, or alternatively, a naive T-cell expressing CXCR4 is first infected by an X4 variant and after differentiation into a memory cell with upregulation of CCR5, super-infected by a R5 variant. The continuous divergence of envelope gp120 sequences suggests that only the full R5 and full X4 sequences have sufficient fitness to persist, counter selecting potentially emerging env-recombinants.

About 50% of HIV infected individuals progress to AIDS in the presence of only R5 HIV-1 variants. However, R5 HIV-1 variants have also been associated with long term non progression (LTNP). We studied potential differences between R5 HIV variants that were isolated early and late in the clinical course of infection from LTNP and progressors. As compared to R5 HIV variants isolated late in infection, the R5 viruses obtained early in infection were less cytopathic, replicated to lower maximum levels, and were more susceptible to inhibition by RANTES, the natural ligand of CCR5. The increase in cytopathicity, virus production and resistance to RANTES was significant in progressors but not in LTNP. This suggests that R5 HIV-1 evolution may contribute to a progressive disease course.

HIV-1 neutralization sensitivity

We previously described the adaptation of the neutralization-sensitive HIV-1 strain IIIB to a neutralization-resistant phenotype in an accidentally infected laboratory worker. During long term propagation of this resistant isolate, designated FF3346, on primary peripheral blood leukocytes *in vitro* an HIV-1 variant appeared that had regained sensitivity to neutralization by soluble CD4 (sCD4) and the broadly neutralizing monoclonal antibody b12. When an early passage of FF3346 was subjected to limiting dilution culture in peripheral blood mononuclear cells, 8 virus variants were isolated with varying neutralization resistance. Two of them, the sCD4 neutralization-resistant variant LW_H8^{res} and the sCD4 neutralization-sensitive variant LW_G9^{sens}, were selected for further study. Interestingly, these two viruses were equally resistant to neutralization by agents that recognize domains other than the CD4-binding site.

Initiation of a potent five-drug, triple class regimen during primary HIV-1 infection does not result in virus-specific immune control upon discontinuation of therapy after 44 weeks.

Site-directed mutagenesis revealed that the increased neutralization sensitivity of variant LW_G9^{sens} results from only two changes, an Asn to Ser substitution at position 164 in the V2 loop and an Ala to Glu substitution at position 370 in the C3 domain of gp120. In agreement with this notion, the affinity of b12 for monomeric gp120 containing the N164S and A370E substitutions in the background of the molecular clone LW_H8^{res} was higher than its affinity for the parental gp120. Surprisingly, no correlation was observed between CD4 binding affinity for monomeric gp120 and the level of neutralization resistance, suggesting that differences in sCD4 neutralization sensitivity between these viruses are only manifested in the context of the tertiary or quaternary structure of gp120 on the viral surface. The results obtained here indicate that the neutralization-sensitive strain IIIB can become neutralization-resistant *in vivo* under selective pressure by neutralizing antibodies, but that this resistance is easily reversed in the absence of immunological pressure.

The viral reservoir in acute infection

Starting standard antiretroviral therapy within 10 days after the onset of a primary HIV-1 infection cannot prevent the establishment of a reservoir of HIV-1-infected memory CD4 T-cells. Here we studied the reservoir of HIV-1-infected memory CD4 T-cells in four patients who started a triple class, five-drug regimen during primary HIV-1 infection. There was a strong correlation between the proportion of productively infected CD4 HLA-DR- T lymphocytes and plasma HIV-1 RNA levels ($r=0.852$; $P<0.001$) during the first 24 weeks of therapy. Within 45 weeks of treatment, in three of the four patients the proportion of productively infected CD4 HLA-DR- T lymphocytes was reduced below the level of quantification. In the fourth patient the cellular reservoir remained quantifiable. In two patients who stopped therapy 44 weeks after initiation an immediate rebound of the plasma HIV-1 RNA level and the proportion of productively infected CD4 HLA-DR- T lymphocytes occurred. In conclusion, initiation of a potent five-drug, triple class regimen during primary HIV-1 infection does not result in virus-specific immune control upon discontinuation of therapy after 44 weeks. Therefore, longer or even stronger suppression of viral replication might be necessary to achieve this goal in primary HIV-1 infection.

Key publications

Beaumont T, Quakkelaar E, van Nuenen A, Pantophlet R, Schuitemaker H. Increased sensitivity to CD4 binding site-directed neutralization following *in vitro* propagation on primary lymphocytes of a neutralization-resistant human immunodeficiency virus IIIB strain isolated from an accidentally infected laboratory worker. J Virol. 2004; 78(11):5651-7.

Stalmeijer EH, Van Rij RP, Boeser-Nunnink B, Visser JA, Naarding MA, Schols D, Schuitemaker H. *In vivo* evolution of X4 human immunodeficiency virus type 1 variants in the natural course of infection coincides with decreasing sensitivity to CXCR4 antagonists. J Virol. 2004; 78(6):2722-8.

Post-entry restriction of the HIV-1 based lentiviral vector

Principal investigator
Neeltje Kootstra MD PhD Dept Clinical Viro-Immunology (n.kootstra@sanquin.nl)

As a natural defense against retroviruses, human and non-human primate cells express inhibitory factors. The mechanism of this retroviral restriction shares many characteristics with the restriction observed for murine leukemia virus (MLV) by Fv-1 in murine cells: The restriction occurs at an early post-entry step; the restricting factor can be saturated with large amounts of virus; and the viral target for the inhibitor is located in the gag capsid protein. In human cells, infection of N-tropic MLV is blocked by inhibitory factor Ref-1, whereas infection with B-tropic MLV and primate lentiviruses is unaffected. In non-human primate cells, the inhibitory factor Lv-1 has recently been identified to be Trim5α, and restricts infection of N-tropic MLV and the primate lentiviruses SIV and HIV-1. The mechanism by which these cellular factors are able to inhibit early steps in the replication cycle of specific retro- and lentiviruses is yet unknown. Recently, we observed that the HIV-1 based lentiviral vector is restricted at an early post-entry step in non-human primate cells due to the presence of an inhibitory cellular factor (Lv-1). When the cyclophilin A (CyPA) binding region in the gag capsid in the packaging construct of the HIV-1 vector was substituted with that of the macrophage tropic HIV-1 Ba-L, the vector became resistant to Lv-1 and efficiently infected simian cells. The alterations in the CyPA binding region did not effect CyPA incorporation, however in contrast to the wild type lentiviral vector infection with the gag chimeric lentiviral appeared to be independent of CyPA indicating that it became also resistant to Ref1, the inhibitory factor present in human cells. In dual infection experiments, the chimeric HIV-1 vector failed to remove the block to infection of the

Although the mechanism of Ref1 and Lv1 mediated inhibition of HIV-1 is still unknown, we believe that the cellular protein CyPA plays a key role.

wild-type HIV-1 vector, suggesting that the chimeric HIV-1 vector did not bind Lv-1. HIV-1 group O and HIV-2 are highly related to HIV-1 (group M) and similar to group M HIV-1, the capsid protein of these viruses are able to incorporate CyPA into the virion. When the CyPA binding region of HIV-1 group O and HIV-2 was placed into the HIV-1 group M backbone, the virus became resistant to Ref1, however resistance to Lv1 was only observed for the virus that contained the CyPA binding region of HIV-2.

Although the mechanism of Ref1 and Lv1 mediated inhibition of HIV-1 is still unknown, we believe that the cellular protein CyPA plays a key role and this is currently under investigation.

Key publications

Kootstra NA, Matsumura R, Verma IM. Efficient production of human FVIII in hemophilic mice using lentiviral vectors. Mol Ther. 2003; 7(5 Pt 1):623-31.

Kootstra NA, Munk C, Tonnu N, Landau NR, Verma IM. Abrogation of postentry restriction of HIV-1-based lentiviral vector transduction in simian cells. Proc Natl Acad Sci U S A. 2003; 100(3):1298-303.

Quality, safety and efficiency

Pathogen detection and inactivation

Pathogen inactivation in platelet concentrates

Principal investigator
Arthur J Verhoeven PhD
Dept Transfusion Technology
(a.verhoeven@sanquin.nl)

In recent years, several techniques to inactivate bacteria and viruses, that might be present in human platelet concentrates, have been evaluated. Preferentially, the use of exogenous compounds for this goal should be avoided in order to protect the recipients of platelet concentrates. In 2003, in collaboration with CAF/DCF (in Brussels) and a third, industrial partner research has started on the possibility to use UV-C light for pathogen inactivation in platelet concentrates. At CAF/DCF, this technique has succesfully been used to treat plasma products. Preliminary results with human platelet concentrates indicate that siginificant virus kill can be obtained with limited damage to the platelets, but much more work needs to done to determine a safe window between pathogen inactivation and platelet damage.

Detection of bacterial contamination of blood products

Principal investigator
Henk Reesink MD PhD Dept
Research and Education
North West Region
(h.reesink@sanquin.nl)

The 16-S ribosomal DNA PCR to detect bacteria in platelet concentrates developed in 2002 in cooperation with the department of microbiology of the VUmc (Head Prof Dr CMJE Vandenbroucke-Grauls) was further investigated. PCR results of platelet concentrates were compared with the results of the automated culturing method (BacT/Alert). At first the investigations were compromised by false positive results due to contamination with bacterial DNA of various reagents. Fortunately a solution to eliminate this problem was found and applied in the further study which will be extended in 2004.

Late 2002 in the Fifth Framework of demonstration projects of the European Commission a project was granted for the development of an impedance measurement to detect bacteria in platelet concentrates. Three (Associate) members of the European Union: Israel (a small company and the Magen David Adom blood center in Tel Aviv), Germany (a company) and The Netherlands (the department of microbiology of the Slotervaart Hospital and the Sanquin Blood Bank North West Region) will cooperate in this project. Sanquin Blood Bank North West region was designated to be the coordinator of the project. In 2003 two prototypes of devices have been

Research for red cell and stem cell sterilisation using porphyrin (Tri-P-4) shows limited red cell damage *in vitro* and sufficient inactivation of non-enveloped viruses and bacteriae.

developed and patented. The investigations in the blood centers and microbiology laboratories will start in 2004. Impedance measurements of platelet concentrates spiked with various species of bacteria will be compared with conventional culturing.

Key publication

Vrielink H, Reesink HW. HTLV I/II prevalence in different geographic locations. Transfus Med Rev 2004; 18(1):46-57.

Photodynamic sterilization of blood products

Principal investigator
Prof Anneke Brand MD PhD Dept Research and Education South West Region (anneke.brand@bloodrtd.nl)

The aim of this research explores the clinical application of inactivation of undesired micro-organisms in blood products and the consequences for cell functions, reduction of cell damage and effect on immuno-modulation.
Research for red cell and stem cell sterilization uses a porphyrin (Tri-P-4) showing limited red cell damage *in vitro* and sufficient inactivation of non-enveloped viruses and gram – and + bacteriae. Also the viability of cord blood stem cells is hardly affected. We further focus on the observed increase in IgG binding on Tri-P-4 treated red cells, immuno-modulation and stem cell viability. Interestingly proliferative lymphocyte functions are not affected by PDT, while the proliferative response on allogeneic cells and stimulator capacity in mixed lymphocyte cultures is impaired. The level of this defect seems located at the cell membrane and its nature is further studied. Collaboration: LUMC dept of IHB (D Roelen).

S-59 platelet inactivation (EuroSPRITE)

Principal investigator
Prof Dick van Rhenen MD PhD Dept Research and Education South West Region (dick.van.rhenen@bloodrtd.nl)

The Blood Bank South West Region participated in a European study on the effect of S-59 platelet inactivation In collaboration with Baxter Healthcare and Cerus Corporation (euroSPRITE study *in vitro* and *in vivo* research was conducted to the safety and effectiveness of transfusions with S59 photochemically treated platelets). Photochemical treatment inactivates the DNA/RNA present in the blood product and this inactivates the viruses, bacteria, protozoa and leukocytes. The results are promising. A national study to investigate the performance of S-59 platelets in clinical practice, is in preparation. Collaboration: Baxter Health Care Corp, Cerus Corp.

Assays were developed for the protein EDRF, which is considered to be a surrogate marker for prion disease.

Key publication

van Rhenen D, Gulliksson H, Cazenave JP, Pamphilon D, Ljungman P, Kluter H, Vermeij H, Kappers-Klunne M, de Greef G, Laforet M, Lioure B, Davis K, Marblie S, Mayaudon V, Flament J, Conlan M, Lin L, Metzel P, Buchholz D, Corash L. Transfusion of pooled buffy coat platelet components prepared with photochemical pathogen inactivation treatment: the euroSPRITE trial. Blood. 2003; 101(6):2426-33.

Prion research

Principal investigator
Frank van Engelburg
PhD Dept Clinical Viro-Immunology
(f.vanengelenburg@sanquin.nl)

Aim of this research is to develop assays for infectious prions in blood and to develop assays to monitor the behavior of prion protein in the production process. Previously an assay for the infectious prion protein was developed. In cooperation with the Department of Viral Safety Services this assay was further optimized. Initial experiments indicated that the assay can be used for monitoring *in vitro* infection of neuroblastoma cell lines. Furthermore, together with the department of Experimental Immunohematology, assays (PCR as well ELISA) were developed for the protein EDRF, which is considered to be a surrogate marker for prion disease. Currently a panel of healthy donors is tested to establish the normal range for these parameters.

Improving materials and methods for blood bank processing

Flowcytometric analysis of leukocytes and leukocyte fragments

Principal investigators
Janny de Wildt-Eggen
PhD Dept Research and Education North East Region
(j.dewildt@sanquin.nl)
Pieter F. van der Meer
PhD Dept Research and Education North West Region
(p.vandermeer@sanquin.nl)

Since the introduction of universal leukoreduction of blood components, the residual number of leukocytes is determined using flow cytometric methods. It was observed that in units that had been stored overnight and subsequently filtered, events were observed that fell outside the set gate, and therefore not counted as leukocytes. In the Netherlands whole blood units can be stored overnight and components must be prepared within 24 hours after collection. The origin and nature of this 'extra' population was unknown, but further analysis revealed that, if these events were included and counted as leukocytes, a large fraction of the blood components would have to be rejected as being leukoreduced. Therefore, in cooperation with Blood Bank North West Region we evaluated the effect of varying whole blood storage times and the use

of different filter systems on the presence of 'extra' populations and their relation to the number of residual white blood cells. Three inline filter systems were used, Baxter, Fresenius and MacoPharma, and were divided in five groups; with various whole blood storage times. White blood cells (inclusive and exclusive 'extra' population) were counted with two flowcytometers (Beckman Coulter (BC) and BD Biosciences (BD)). Standard leukocyte counting procedures were performed using counting kits. This study showed that when all filtered red cell concentrates were prepared within 12 hours of storage time no unit will contain >1x10^6 White blood cells/unit.
The platelets counts of whole blood prepared at the storage time <8 hours were significantly lower compared to those prepared >8 hours. The presence of 'extra' populations was related to the residual white blood cells and varies depending on the filters used and on storage time. Because the major fraction of the total number of leukocytes present in whole blood and red cell concentrates are granulocytes, these results suggest that the extra populations are probably disintegrating granulocytes. Inclusion of the 'extra' population has major effect on the outcomes of quality control measurements, but the clinical relevance of the fragments is uncertain. Further studies are currently carried out to prove this hypothesis.

Key publications

Van der Meer PF, Gratama JW, van Delden CJ, Laport RF, Levering WHBM, Schrijver JG, Tiekstra MJ, Keeney M, de Wildt-Eggen J. Comparison of five platforms for enumeration of residual leucocytes in leucoreduced blood components. Br J Haematology 2001; 115:953-62.

Dijkstra-Tiekstra MJ, van der Meer PF,de Wildt-Eggen J. Multicenter evaluation of two flow cytometric methods for counting low levels of white blood cells. Transfusion 2004; 44(9):1319-24.

Counting residual leukocytes in blood components

Principal investigator
Ruby Pietersz MD PhD Dept Research and Education North West Region (r.pietersz@sanquin.nl)

As a follow up of an evaluation in 2002 it was decided that counting of low numbers of leukocytes on the flow cytometers had to be re-validated. Margriet Dijkstra initiated a national multi-center study by organizing a send around of a panel of blood components (plasma, platelet concentrates and red cells) with various low, 0.3 to 10 x 10^6, leukocytes per unit of 300 ml. Counting on the 2 types of flow cytometer

was performed according to a national protocol. Based on the results, the conclusion was that none of the flow cytometers or methods gave adequate results for all three blood components at the level of 1 x 10^6. However, checking the QC data of routine leuko-reduced components revealed that the counting method could be used as a pass/fail criterion.
Together with Sanquin Diagnostic Services, methods to optimize isolation of (leukocyte-derived) DNA from platelet concentrates and measure free DNA in plasma with a real time PCR technique have been investigated.

Key publication

Dijkstra-Tiekstra MJ, Pietersz RNI, Reesink HW, Vander Schoot CE. Influence of cell-free DNA in plasma on real-time polymerase chain reaction for determination of residual leucocytes in platelet concentrates. Vox Sang. 2004; 86(2):130-5.

Quality of leukocyte-reduced apheresis plasma

Principal investigator
Pieter van der Meer
PhD Dept Research and Education North West Region (p.vandermeer@sanquin.nl)

Various techniques are applied to harvest 'cell free' plasma by apheresis. Recently new software and a new 'high speed core bowl' have been developed by one of the manufacturers. The method required some adjustments of the software but finally adequate results were obtained. Next the composition of the plasma was established, the collection time was compared with the present method. The results revealed that the plasma contained fewer than 10^6 leukocytes and fewer than 108 red cells. The number of experiments was increased to meet the criteria for validation. The method has now been implemented in routine.

Key publication

Vrielink H, Vander Meer PF. Collection of white blood cell-reduced plasma by apheresis. Transfusion. 2004; 44(6):917-23.

Improving materials and methods for storage of blood components

Cryopreservation of RBC and stem cells

Principal investigator
Johan Lagerberg PhD Dept Transfusion Technology (j.lagerberg@sanquin.nl)

Research in the Laboratory of Cryobiology has been limited to the development of a routine method for freezing and subsequent washing of red cells (to remove the cryoprotectant glycerol) in a completely closed system. Based on previous results, obtained in collaboration with the Military Blood Center, a choice was made to gradually change the protocol from a low-glycerol to a high-glycerol method. The latter method allows the processing of a RBC unit in a completelely closed system, allowing the cells to be stored for several days after thawing. This change will facilitate the operations of the Blood Bank for the Council of Europe, in which 1500 units have been frozen to be used for patients with rare blood group antigens. Prior to implementing this change, several parts of the protocol need to be optimized and validated.

RBC storage solutions

Principal investigator
Dirk de Korte PhD Dept Transfusion Technology (d.dekorte@sanquin.nl)

During storage of RCC, several metabolic changes occur. Most significantly, 2,3-DPG (already lowered by 50% after RCC preparation) disappears completely in the first 2-3 weeks of storage, thereby changing the characteristics of oxygen binding to hemoglobin. To prevent this undesired change, we have developed an additive solution based on the 'chloride-shift' principle, allowing maintenance of high 2,3-DPG levels without concurrent ATP decline. In this medium, pH was set at 8.2 and chloride was replaced by gluconate, contributing to an elevated intracellular pH (due to chloride exhange for OH-ions). After encouraging experiments on lab scale, the experimental solution was tested on whole units of RCC using a Haemonetics ACP215 Cell washer. The 2,3-DPG content of RCC washed with experimental solution increased during storage to about 15 µmol/g Hb, which is near the physiological value, and started to decrease after 42 days. ATP content at day 42 was still at its starting value, in contrast to RCC stored in saline containing medium of pH 6.0. Elevation of the medium pH from 6.0 to 8.2 without choride depletion only partially mimicked the effect of the experimental solution. Hemolysis in the experimental solution after 42 days of storage remained well below the limit of 0.8% and only 1% of cells exposed PS (as indicated

by Annexin-V binding). Glucose consumption and lactate production was increased in our experimental solution, but this was not reflected in a lower pH. If directly added to RCC (no washing step to remove resiual plasma containing chloride), the experimental solution showed similar, but less pronounced and less durable effects. Hence, when resuspended in this experimental medium, RCC can be stored for at least 42 days with a 2,3-DPG content similar to that in fresh whole blood, without compromising hemolysis or ATP content. Further studies will be undertaken to test the potential of this approach for transfusion of RCC into patients.

Key publication

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

Platelet metabolism during storage

Principal investigator
Dirk de Korte PhD Dept Transfusion Technology (d.dekorte@sanquin.nl)

Using our newly developed test for the mitochondrial membrane potential in platelets (reported on in 2002), we have investigated the hypothesis that storage-induced lesions in platelet responses might be due to changes in mitochondrial function. Our test is based on the accumulation of the fluorescent dye JC-1 inside the mitochondrial matrix in the presence of a high membrane potential ($\Delta\Psi$) with a concomitant increase change from green to red fluorescence. Thus, after JC-1 loading, the ratio of red (FL2) to green (FL1) fluorescence as determined in a flow cytometer, is an indicator of mitochondrial integrity. During storage of platelet concentrates under standard blood bank conditions, the JC-1 ratio started to decrease after 5 days of storage. This decrease occurred after clear changes in platelet antigen expression had occurred (most notably CD62P), indicating that this effect of platelet storage does not seem to be due to changes in mitochondrial function. In contrast, when platelet concentrates were treated with UV-B light, mitochondrial changes occurred much more rapidly, and under these conditions the change in JC-1 signal strongly correlated with CD62P expression.

Platelet activity changes during storage

Principal investigator
Joyce Curvers PhD Dept Research and Education South East Region (j.curvers@sanquin.nl)

From previous experiments we have learned that platelet products currently produced by blood banks retain their thrombin forming capacity throughout a storage period of 7 days. However, the support of tissue factor driven thrombin generation in these products was insensitive to platelet inhibition. These results suggested that thrombin generation was no longer dependent on the platelets in the product, but more the result of microparticles shed from platelets during storage.

In 2003 a new project concerning the physiological and biochemical changes in platelets upon prolonged storage was started. In this project we have taken a closer look at cell surface changes taking place upon storage under standard blood banking conditions and the effects on platelet biology. We determined activation properties of platelet-plasma preparations that were pooled or collected from single donors via apheresis. It is well described that platelets become less responsive to a wide variety of agonist during storage. In comparison to freshly isolated platelets, both apheresis and pooled platelets exhibited slow exposure of CD62 upon storage, followed by surface appearance of procoagulant phosphatidyl serine. Thrombin and ADP induced calcium signaling decreased in apheresis and pooled platelets during storage and this was accompanied by lower agonist-induced CD62 exposure and activation of the glycoprotein IIb/IIIa. In flowing whole blood, stored apheresis platelets showed diminished participation in thrombus formation and collagen induced calcium responses were lower. The loss in responsiveness could be the result of a loss of functional receptors, due to a diminished or altered signaling that occurs during storage.

This research is performed in collaboration with Synaps BV University Maastricht (P Giesen), and the departments of Biochemistry of University Maastricht (J Heemskerk) and Hematology of University Hospital Maastricht (L van Pampus).

Key publication

Curvers J, van Pampus CM, Feijge MAH, Rombout-Sestrienkova, Giesen PLA, Heemskerk JWM. Decreased responsiveness and development of activation markers of PLTs stored in plasma. Transfusion. 2004; 44: 73-82.

The extension of storage time of platelet pools in plasma from 5 to 7 days was accepted in the Netherlands provided the processing method is validated.

Increments following platelet transfusion

Principal investigator
Ruby Pietersz MD PhD Dept Research and Education North West Region (r.pietersz@sanquin.nl)

In collaboration with the department of Hematology of the VUmc the results of the clinical study to establish platelet increments in stable patients after transfusion of leukocyte reduced platelet concentrates stored in plasma for up to 7 days were further analysed. A slight reduction in increment was observed when increments after transfusion of 2-day-old platelets were compared with those after transfusion of 7-day-old platelets, but the difference was deemed acceptable.
The extension of storage time of platelet pools in plasma from 5 to 7 days has now been accepted in the Netherlands provided the processing method is validated. To gather more data on unexpected adverse reactions and increments in various patients a post authorization surveillance will be performed in 2004.

Key publication

Dijkstra-Tiekstra MJ, Pietersz RNI, Hendriks ECM, Reesink HW, Huijgens PC. *In vivo* PLT increments after transfusions of WBC-reduced PLT concentrates stored for up to 7 days. Transfusion 2004; 44(3):330-6.

Growth factors in platelets

Principal investigator
Joyce Curvers PhD Dept Research and Education South East Region (j.curvers@sanquin.nl)

Besides their role in hemostasis, platelets also release substances that play a role in wound healing and inflammation. The alpha-granula of platelets contain several growth factors such as TGF-β1, IGF-1, VEGF and PDGF. Blood bank platelet products contain on the average about 1000 x 10^9 platelets/L. During storage it is therefore feasible that the concentration of growth factors in platelet products increases due to platelet release reactions.
In a first study we determined the amount of TGF-β1 in platelet-rich and platelet free plasma prepared with the Continuous Autotransfusion System (CATS) of Fresenius Hemocare. In the upcoming year pilot experiments will be performed in which the effects of platelet derived growth factors on the proliferation of several cell types is investigated *in vitro*.

Platelet storage

Principal investigator
Ruby Pietersz MD PhD Dept Research and Education North West Region (r.pietersz@sanquin.nl)

Platelet concentrates are stored with continuous agitation. During transport this agitation will be interrupted. An international protocol from the BEST (Biological Excellence for Safer Transfusion) group to assess interruption of agitation from 24 to 96 hours was followed to investigate storage parameters *in vitro*. We studied leukocyte reduced platelet pools from 5 buffy coats and stored in two different platelet additive solutions: PAS III modified and Composol, respectively in one type of platelet storage bag. Two platelet concentrations were studied: a 'normal' concentration of about 1 x 10^9 platelets/ml and a 'high' concentration of about 2 x 10^9 platelets/ml. The results revealed that interruption of agitation for 24 hours did not negatively influence storage parameters for any storage medium or concentration. In PAS III modified the platelets had acceptable storage parameters even when the agitation was interrupted for up to 96 hours, provided the platelets were stored with a 'normal' concentration.
To investigate the influence of sterilization on the material of platelet storage bags we investigated bags with butyl-tri hexyl-citrate (BTHC) as plasticizer either steam sterilized ('wet' sterilized) or with ethylene oxide (ETO) ('dry' sterilized). One size bag from various manufacturers was studied. This size of the bag is intended for storage of 250 to 500 x 10^9 platelets either from pools of 5 buffy coats or obtained via apheresis. All bags were adequate to store leukocyte-reduced platelet concentrates for up to 7 days.
As part of the Sanquin project to investigate 'hibernation' of platelets by absence of glucose or low temperatures experiments were started by measuring parameters *in vitro* of platelets in absence of glucose stored at temperatures between +4°C and +37°C. After defined storage periods glucose was added; the platelets showed adequate recovery. Further experiments will focus on storage at +4°C, prevention of changes in the platelet surface due to the low temperature and development of a test to predict the removal of platelets in the body. The study is performed in collaboration with the Department of Thrombosis and Hemostasis of the Utrecht Medical Center (Head Prof JW Akkerman).

New therapies and evaluation of clinical applications

Stem cell research

Homing of hematopoietic progenitor cells

Principal investigators
Ellen van der Schoot MD PhD Dept Experimental Immunohematology (e.vanderschoot@sanquin.nl)
Peter Hordijk PhD Dept Experimental Immunohematology (p.hordijk@sanquin.nl)
Jaap-Jan Zwaginga MD PhD Dept Experimental Immunohematology and Academic Medical Center Univ. Amsterdam (j.zwaginga@sanquin.nl)
Carlijn Voermans PhD Dept Experimental Immunohematology (c.voermans@sanquin.nl)

The homing of hematopoietic progenitor cells (HPCs) is one of our main research lines. Last year an *in vivo* model for stem cell homing has been developed by transfecting the CXCR4-negative CD34-positive KG1a cell line with CXCR4, and transplanting these cells into NOD-SCID mice. In this way we have shown that CXCR4 is essential for stem cell homing. A new project started directed at the application of mesenchymal stem cells as support for stem cell transplantation. New research will be focused on the potential of blood or bone marrow derived cells for therapeutic neovascularisation.

Previous work has shown that stem cells can be expanded *ex vivo* into megakaryocytes by the combination of thrombopoietin (Tpo) and IL-1. In the near future cancer patients will be treated with expanded megakaryocytes in a MEC-approved trial (in collaboration with Prof Rodenhuis and Dr Baars, AvL/NKI, Amsterdam). Additionally, in animal models we will test the homing of the *ex vivo* expanded megakaryocytes to bone marrow, and we will try to optimize this essential process.

The studies on the role of VE-cadherin as an important factor in the efficiency of HPC transendothelial migration have been continued. Recent work has focused on intracellular signaling that is initiated upon loss of cadherin function.

Key publications

Van Buul JD, Voermans C, van Gelderen J, Anthony EC, van der Schoot CE, Hordijk PL. Leukocyte-endothelium interaction promotes SDF-1-dependent polarization of CXCR4. J Biol Chem 2003; 278(32): 30302-10.

Beillard E, Pallisgaard N, van der Velden VH, Bi W, Dee R, van der Schoot E, Delabesse E, Macintyre E, Gottardi E, Saglio G, Watzinger F, Lion T, van Dongen JJ, Hokland P, Gabert J. Evaluation of candidate control genes for diagnosis and residual disease detection in leukemic patients using 'real-time' quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR) – a Europe against cancer program. Leukemia 2003; 17(12):2474-86.

We have shown that *in vitro* megakaryocyte progenitor expansion is feasible in patients who experienced prolonged thrombocytopenia after stem cell transplantion.

Principal investigator
A Lyndsay Drayer PhD Dept Research and Education North East Region (l.drayer@sanquin.nl)

Factors affecting proliferation and differentiation of stem cells

In cancer patients receiving high-dose chemotherapy and hematopoietic stem cell (HSC) transplantation the period of profound cytopenia generally lasts between 1 to 6 weeks, depending on the number and source of HSCs infused. Supplementing stem cell transplants with *ex vivo* expanded progenitor cells may be an approach to accelerate the hematopoietic recovery. In the human setting, multiple studies have now demonstrated that the *ex vivo* expansion process can be used to generate large quantities of more mature progenitor cells, and a number of clinical studies have shown promising results. In our *ex vivo* expansion studies we have focused on expansion of the megakaryocytic lineage, as the period of thrombocytopenia following stem cell transplantation is a major problem for many patients. We have shown that *in vitro* megakaryocyte progenitor expansion is feasible in patients who experienced prolonged thrombocytopenia after stem cell transplantion. The aim during the *ex vivo* expansion process is to stimulate the proliferation phase to generate a maximal yield of progenitors, while at the same time limiting differentiation to mature, non-proliferating cells. In addition, the cells need to preserve the capacity to migrate and engraft in the bone marrow.

Dissecting the cross talk between cytokines involved in proliferation (to generate many progeny) and differentiation (to generate cells at a specific maturation stage with the appropriate homing properties) should prove to be valuable in uncoupling the proliferation and differentiation processes during expansion culture. In present studies we have analysed how cytokines interact at the cellular and molecular level by investigating their effects on proliferation, differentiation and signal transduction processes in megakaryocte and erythroid progenitor cells. In a recent study we described the cross talk between Erythropoietin (Epo) and Stem Cell Factor (SCF)-activated signal transduction pathways by analysing their effects on STAT5 signaling (ref 2,3). We demonstrated that the synergistic effects between SCF and Epo on erythropoiesis might be regulated by an enhancing effect of SCF on Epo-mediated STAT5 transactivation. In ongoing studies we are investigating the effects of SCF during megakaryocyte expansion. As SCF is widely used in expansion protocols, it is of interest to study the effect of stem cell factor on activation or down-regulation of specific signaling

pathways linked to proliferation or differentiation. In future experiments we will examine the role of individual genes during proliferation and differentiation by RNA interference techniques.
These projects are performed in collaboration with Prof E Vellenga, Dept of Hematology, University Hospital Groningen.

Key publications

Drayer AL, Smit Sibinga CT, Esselink MT, de Wolf JT, Vellenga E. *In vitro* megakaryocyte expansion in patients with delayed platelet engraftment after autologous stem cell transplantation. Ann Hematol. 2002; 81(4):192-7.

Boer AK, Drayer AL, Vellenga E. Stem cell factor enhances erythropoietin-mediated transactivation of signal transducer and activator of transcription 5 (STAT5) via the PKA/CREB pathway. Exp Hematol 2003; 31(6):512-20.

Boer AK, Drayer AL, Vellenga E. cAMP/PKA-mediated regulation of erythropoiesis. Leuk Lymphoma 2003; 44(11):1893-901. Review.

Development of dendritic cell-based cellular transfusion product for immunotherapy of renal cell carcinoma and metastatic melanoma patients

Principal investigator
Mona Elias MD PhD Dept Research and Education North East Region (m.elias@sanquin.nl)

Patients with metastatic renal cell carcinoma and metastatic melanoma do have a poor prognosis without an effective treatment. These tumour types are known to be immunogenic due to sporadic occurrences of spontaneous disappearance of tumour nodules. The rationale to develop an anti-tumour vaccine for patients with these malignancies metastatic renal cell carcinoma and metastatic melanoma is based on this immunogenic profile. Dendritic cells (DCs) are the most potent antigen presenting cells (APCs) as they contain all that is needed to activate memory and naïve T-cells including high MHC class I and class II expression, in addition to the presence of adhesion molecules and accessory receptor structures of the B7 family and

the secretion of multiple soluble factors. Presentation of tumour antigen by APCs is crucial for the induction of an antigen-specific CTL response. Clinical trials using *ex vivo*-pulsed DCs have been shown to induce antigen-specific CTL response against tumour antigens and tumour regression in some vaccinated patients. Based on these findings, the Department of Medical Oncology of the University Hospital Groningen started, a Medical Ethical Committee-approved phase I study with a tumour vaccine consisting of either an autologous or an allogeneic fusion product (donor monocytes with different HLA phenotypes and autologous tumour cells). The procedure performed in two patients proved to be non-toxic. The outcome, however, indicated that the antigen delivery in the used fusion model was most likely sub-optimal. In addition, the technology applied was cumbersome and not applicable to a large scale. This triggered a project to design DC-based transfusion vaccine to be realized in three phases, starting with large-scale production of clinical-grade dendritic cells. The second phase focuses on selection of the optimal DC loading strategy that induces most efficiently an antigen-specific immune response. Antigen delivery by electroporating genetic material (mRNA) encoding the antigens of interest into DCs to provide them with a renewable source of antigen for presentation will be investigated. This non-viral DC transfection technique will be compared to cross priming by uptake of apoptotic tumour cells. When the feasibility and efficiency of either approach is proven, the cellular vaccine will be up scaled for clinical use and validated in a clinical vaccination trial.

We have developed conform GMP guidelines a clinical grade protocol for generation of stable DCs using a closed-system all through collection of mononuclear cells, isolation of monocytes, culture of DCs and cryopreservation. The quality of the initial mononuclear cell product was found to determine the outcome of monocyte enrichment. Enriched monocytes were cultured in a new generation gas permeable containers under serum-free conditions with GM-CSF and IL-4. A seeding concentration of 1×10^6 was found optimal in terms of DC phenotype expression, monocyte percentage in culture and cell viability. The differentiation pattern favours day 7 for harvest of immature DCs. DCs were induced to maturation using LPS versus a cytokine cocktail of IL1β, TNFα, PGE2, and IL6. In contrast to the latter, serum was

Ex vivo expanded cord blood cultures with trombopoietin do not only resulted in earlier platelet engraftment but also in normal or better engraftment of the other hematopoietic cells.

found indispensable when LPS was solely used as maturation agent. Mature serum-free DCs expressed the CD83 marker with significant upregulation of costimulatory molecules and HLA-DR and were able to generate an allogeneic T-cell response as well as an anti-CMV response in proliferation assays. DCs derived from a single volume of blood processed as described in this protocol, are quantitatively and qualitatively fulfilling the criteria required for clinical application in immunotherapeutic protocols.

Cord blood

Principal investigator
Prof Anneke Brand MD PhD Dept Research and Education South West Region (anneke.brand@bloodrtd.nl)

The purpose of this research is to increase the applications of placental blood for transplantation and transfusion. This includes expansion of stem cells and exploration of conditions to improve of engraftment in order to facilitate transplantation in adult patients. In addition, expansion of progenitor cells, immunological profile of cord blood mononuclear cells and placental red cells are evaluated for transfusion purposes.
Research includes expansion of megakaryocyte precursor cells. We characterized that in contrast to bone marrow and peripheral blood mobilized stem cells, that the $CD34^{+}/41^{neg}$ subset in cord blood contributes to 99% of *in vitro* generated megakayocyte precursors. This was confirmed by *in vivo* engraftment of platelets in the NOD/SCID mouse by transfusion of distinct subpopulations of *in vitro* expanded megakaryocyte progenitors. Moreover we observed that *ex vivo* expanded cord blood cultures with trombopoietin not only resulted in earlier platelet engraftment but also in normal or even better engraftment of the other hematopoietic cells. Currently we study the effect of co-culture of cord blood stem cells with mesenchymal stem cells (MSC's) further enhances engraftment potentials and characterize whether distinct homing patterns exist for expanded and non-expanded CD34 cord blood cells. This work is performed in collaboration with the Dept of Exp Hematology LUMC (WE Fibbe).

In collaboration with the group from Prof E Goulmy (Dept of Immunohematology, LUMC), the effects of *ex vivo* expansion on the immune competence of cord blood cells (relevant for Graft versus Leukemia effects) will be studied.

In 2002 we finished a pilot study on cord blood collection of premature children. This study was undertaken to investigate the possibility to use autologous placental red cells for transfusion to premature infants. The results show that a red cell product can be prepared according to Blood Bank standards and can be stored for at least 2 weeks. In 2004 a clinical trial will start to investigate feasibility and possible advantages of these transfusions. In addition the autologous erythropoietic progenitor cells will be expanded for possible transfusion purposes. The autologous cord blood project is integrated in a medical student project who participate in a 24 hour on call system for cases of premature delivery (Collaboration Leiden University Medical Center: HHH Kanhai, S Scherjon, Dept of Obstetrics, F Walther, Dept of Neonatology, L Christiaens, Dept of Obstetrics UMCU, Prof H Brouwer, Dept of Neonatology UMCU).

Research on cellular blood products

Use of autologous erythrocytes products collected with component collection (double portion erythrocytapheresis)

Principal investigator
Eva Rombout MD Dept Research and Education South West Region (e.rombout@sanquin.nl)

Objectives of this study are (i) to analyse the cost effectiveness of collection of autologous erythrocytes by means of whole blood donation versus erythrocytapheresis; (ii) to evaluate whether the collection of double portion erythrocytes reduces the use of additional homologous blood products; and (iii) to evaluate the number of donor complications during this type of autologous blood collection.
We already have evaluated 23 first donors who underwent a total of 33 procedures. Judged by the initial blood pressure and pulse rate and their changes during the procedure, this method did not seem to be a stressful event for these donors. Only in five procedures donors had complaints (three concerning a slight citrate reaction and two concerning dizziness shortly after the procedure). All complaints disappeared without interference. At this moment we can conclude that double erythrocytapheresis seems a feasible, well tolerated, safe and efficient procedure to collect autologous erythrocytes. The cost effectiveness will be compared in due course.
This project is performed in collaboration with Atrium Medical Center Heerlen, Maasland Hospital Sittard, Laurentius Hospital Roermond and Vicuri Hospital Venlo.

Pilot data pointed out that donor characteristics might determine the amount of single donor platelets.

The effectiveness of therapeutic erythrocytapheresis in treatment of Hemochromatosis

Principal investigator
Eva Rombout MD Dept Research and Education South West Region (e.rombout@sanquin.nl)

Phlebotomy is the classical treatment in severe cases of primary hemochromatosis. Depending on the required reduction in iron overload, 25 to 100 blood lettings are required over a period of six to 24 months. The maximum number of treatments per year is limited because of the concomitant blood loss. Erythrocytapheresis allows a more intense treatment since erythrocytes can be selectively removed from the circulation. This erythrocytapheresis procedure is claimed to be both more patient-friendly as well as more effective, that is to say in intensity per intervention as well as by limiting the number of required interventions. A pilot project in the blood bank location in Limburg showed that in about seven instrumental erythrocytapheresis procedures over a period of up to three months the mean serum ferritin level decreased from 1690 to 67 microgram/l.

In this project we plan to 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'. Aspects of cost effectiveness will be included in the final analysis. The results of the study would allow decision-making based on Evidence Based Medicin on the 'best' therapeutic options available for newly-diagnosed as well as existing primary hemochromatosis patients. This project is performed in collaboration with Atrium Medical Center Heerlen and University Hospital Maastricht.

Conversion to single donor platelets; donor selection criteria to maximize double platelets products (dpp) by thrombocytapheresis

Principal investigator
Jacques Wollersheim MD Dept Research and Education South West Region (j.wollersheim@sanquin.nl)

The emergence of the variant Creutzfeldt-Jakob as a disease possibly transmitted by blood transfusion, induced the Medical Advisory Board of Sanquin (The Netherlands) to explore measures to reduce donor exposure by apheresis platelets. From 2002 a pilot study investigated the conversion from buffy coat platelets to single donor platelets. The first 100 procedures showed that it was technically possible to increase the amount of single donor platelets to 176. Conversion would be logistically feasible if donors are willing to donate at least eight times a year.

The pilot data pointed out that donor characteristics might determine the amount of single donor platelets, yet needed further analysis. The logistical assumption still had to be confirmed by the willingness of the donors. The current study aimed to develop

In open heart surgery there is a decreased occurence of infections and lower mortality with multi-organ failure in the group which received leukoreduced RBC.

donor selection criteria that maximize the likelyhood of deriving single donor platelets. Donor precount and body weight were examined as determinants of higher split-rates combined with procedure time and the willingness of these donors to donate at least eight times a year. Thrombocytapheresis donors were recruited among plasmapheresis donors by historical platelet precount >250x10^9/l and weight >70 kg, and among other donors when the selected donors did not show up. Procedures were performed with Trima Accel (Gambro BCT).

In order to maximize the likelyhood of deriving single donor platelets, both precount and body weight appeared useful selection criteria with precount being more predictive of yielding DPP. The cut-off point was preferably set on precount of >225x10^9/l and 65 kg. The reported donor willingness on donation frequency confirmed the logistical feasibility of the conversion to single donor platelets. This project was performed in collaboration with 13 Hospitals in region Brabant.

Leukoreduction of blood products

Principal investigators
Prof Anneke Brand MD PhD Dept Research and Education South West Region (anneke.brand@bloodrtd.nl)
Joost van Hilten PhD Dept Research and Education South West Region (Joost.vanHilten@bloodrtd.nl)

In 2003, the results of two multicenter randomized studies on leukoreduction of red cell products were finalized. One study included major vascular and gastro-intestinal surgery, the other open heart surgery. Most obvious results in open heart surgery are a decreased occurence of infections and lower mortality with multi-organ failure in the group which received leukoreduced RBC. In major vascular surgery less patients developed multi-organ failure when leukoreduced RBC were used and length of stay in hospital was significantly shortened.

Currently a meta-regression analysis is performed of all patients who participated in randomized trials on this subject since 1992. Also genetic and environmental factors, which are presumed to contribute to development, severity and recovery of MODS are studied to unravel the mechanism.

Key publication

Van Hilten JA, van de Watering LM, Van Bockel JH, van de Velde CJ, Kievit J, Brand R, Hout WB, Geelkerken RH, Roumen RM, Wesselink RM, Gemert AW, Koning J, Brand A. Effects of transfusion with red cells filtered to remove leucocytes: randomized controled trial in patients undergoing major surgery. Br Med J 2004; 328:1281.

Reduction of blood transfusion in orthopedic surgery

Principal investigator
Prof Anneke Brand MD PhD Dept Research and Education South West Region (anneke.brand@bloodrtd.nl)

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 transfusions, wound blood, large multi-arm and multicenter studies are needed. The basic requirement for such studies is a strict transfusion protocol. We finished a trial in three hospitals (Leiden University Medical Center, Leyenburg Hospital, and Reinier de Graaf Hospital) to compare a fixed restrictive transfusion trigger with common practice in the hospital in patients undergoing hip and knee surgery. The results revealed that among hospitals a generally accepted transfusion trigger is feasible, which can serve as control arm for new studies.

Transfusion triggers and effects

Transfusion effects in myelodysplastic patients: limiting exposure (TEMPLE)

Principal investigator
Prof Dick J van Rhenen MD PhD Dept of Research and Education South West Region (dick.van.rhenen@bloodrtd.nl)

In ambulant hematological patients generally a hemoglobin level above 5 mmol/L is maintained to improve Health-related Quality of Life (HRQoL).
In fifty randomly chosen patients with MDS (Myelodysplastic syndromes) we investigated the effect of anemia on objective symptoms of fatigue). The questionnaires have a high feasibility and MDS patients have a worse HRQoL than age-related persons. There is a correlation found between hemoglobin level and HRQoL, especially the physical domain. Both hemoglobin value as HRQoL seem to be relevant for evaluation of the severity of chronic anemia. The optimal transfusion trigger in patients with MDS is currently evaluated in a prospective randomized clinical trial. In this study a trigger of 4.5 mmol/l hemoglobin is compared with a trigger of 6 mmol/L. Primary outcome is fatigue, measured with the MFI questionnaire. The goal of this temple study is to develop a new HRQoL driven transfusion policy.

Health related quality of life based transfusion policy

Optimal transfusion trigger in patients with Acute Myeloid Leukemia (AML). Red-cell transfusions are the cornerstone of supportive care for hematological patients treated with intensive chemotherapy. Concerns about the transfusion-related complications,

such as infections, tumour behavior and immunomodulatory effects, and the costs, necessitated a re-evaluation of the transfusion practice. We finished a retrospective analysis in 84 patients with AML, who were treated with intensive chemotherapy. We observed that a restrictive policy of red-cell transfusion (4.5-5.5 mmol/l, dependent on patient age and symptoms, n=38) had led to a diminished use of red-cell transfusions compared to a liberal transfusion policy (i.e. transfusion if Hb ≤6 mmol/l, n=46). We found no difference in safety , bleeding risks or effectiveness of chemotherapy to induce leukemia remission.

Another group of patients in which transfusions are often administered with the presumption to improve HRQoL are females with post-partum anemia due to bleeding. In a pilotstudy three internationally used HRQoL questionnaires (SF36, EuroQoL and MFI) are validated in fifty patients. Also the relationship HRQoL and anemia will be measured in this cross-sectional design. The questionnaires have been used to design a prospective randomized clinical trial to develop a more objective HRQoL driven transfusion policy. In this study 200 patients are either assigned to a restrictive or a liberal transfusion trigger. Primary outcome is HRQoL. The goal of the study is to develop a more HRQoL based transfusion policy based on the HRQoL questionnaires, validated in a pilot study.

Key publication

Jansen AJ, Essink-Bot ML, Beckers EA, Hop WC, Schipperus MR, Van Rhenen DJ. Quality of life measurement in patients with transfusion-dependent myelodysplastic syndromes. Br J Haematol 2003; 121(2):270-4.

Well-being of obstetric patients on Minimal Blood transfusions (WOMB study)

In a multicenter randomized clinical trial 400 patients will be included (200 patients after vaginal delivery and 200 patients after cesarean section). Inclusion criteria are 12-24 hours postpartum, a blood loss of at least 1000 ml and a Hb value between 3.0 and 4.9 mmol/l. In both groups 100 patients will receive a red cell blood transfusion and 100 patients won't receive red blood cell transfusion. Primary outcome is Health Related Quality of Life. The goal of the study is to develop a more HRQoL based transfusion policy for patients after delivery, based on the HRQoL questionnaires, validated in the pilotstudy.

Donor studies, epidemiology and cost effectiveness

Principal investigator
Cees van der Poel MD
PhD Sanquin Research
(c.vanderpoel@sanquin.nl)

Transfusion Technology Assessment (TTA)

While blood products for transfusion are safe, technology becomes available to create even more safe transfusion products. As blood is human derived, it is exposed to (ever changing) infectious diseases. Therefore blood transfusion can never be totally safe. On the one hand this creates opportunities for 'marketing of fear'; on the other hand there is a need for efficacy parameters for safety measures. In collaboration with the department for Medical Technology Assessment (MTA) of the Julius Center of Utrecht University, Sanquin Research created the research group on Transfusion Technology Assessment (TTA). The group aims to evaluate independently safety issues in blood transfusion. Of importance are risk analyses, costs and effects of safety interventions, user statistics of blood and blood products, and the profile of the blood recipient.

Risk analyses in the field of blood transfusion were done based on literature and corporate data from Sanquin. Results were reported to Sanquin and forwarded by the Sanquin Executive Board to the Ministry of Health. As will be done annually in the future.

Charting blood use and profiles of recipients of blood products in the Netherlands was done by developing a set of parameters. Participation of hospitals is of course essential. Therefore the TTA group duly established collaboration with the Dutch Haemovigilance Organization TRIP.

In 2003, a cost effectiveness study on pathogen reduction vs. bacterial screening was started. Commissioned by the Council of Europe, indicators of the use of blood products in Europe were reported in November 2003 and evaluated, including proposals to improve the methodology used. Publication of the results is expected in 2004 after revision by member states. Annual updates are to be expected.

Due to the recent start of this research group, its first publications will be submitted to journals in 2004. A publication on blood transfusion specific technology assessment for a larger audience is in preparation as well.

Analysis of disease markers in patients who were blood donor in the past

Principal investigator
Henk Reesink MD PhD Dept Research and Education North West Region (h.reesink@sanquin.nl)

In collaboration with the Jan van Breemen Institute (Head Prof BAC Dijkmans) a study was started in 2002 to investigate markers for rheumatoid arthritis (RA) in patients who had been donors in the then Blood Bank North Holland (Amsterdam). The patients/donors gave their consent, as much samples as possible were looked up in the 'bank' of stored frozen samples of donations. Anonymous matched controls were also identified and searched for. In all samples RA markers were tested. Increased levels of C-Reactive Protein were observed within 2 years before the onset of symptoms.

Key publication

Nielen MM, van Schaardenburg D, Reesink HW, Twisk JW, van de Stadt RJ, van der Horst-Bruinsma IE, de Gast T, Habibuw MR, Vandenbroucke JP, Dijkmans BA. Increased levels of C-reactive protein in serum from blood donors before the onset of rheumatoid arthritis. Arthritis Rheum 2004; 50(8):2423-7.

Blood donation, iron status and atherosclerosis

Principal investigator
Tini Hoekstra PhD Dept Research and Education South East Region (t.hoekstra@sanquin.nl)

In several epidemiological studies it was shown that blood donation is associated with a reduced cardiovascular risk, which might be mediated by a reduction in body iron load. It has been hypothesized that iron may promote atherosclerosis by the formation of oxygen radicals. In a cross-sectional study among 819 healthy men and women, (50-70 years of age), the associations between blood donation, iron status and atherosclerosis were investigated. The subjects were recruited from Sanquin Blood Bank South East Region and city registers. Thickness of the intima-media layer (IMT) of the common carotid artery was measured as a marker of generalized atherosclerosis. As expected, blood donor status was associated with most iron parameters (ferritin, non-transferrin bound iron (NTBI), transferrin saturation (TS) and total iron-binding capacity (TIBC)). Serum iron did not significantly differ between current or ex-donors, and never between blood donors. Neither blood donation (duration, frequency) nor iron parameters were consistently associated with IMT. Thus, in this study no evidence was found for the hypothesis that blood donation lowers the risk for cardiovascular disease. This project was performed in collaboration with the Division of Human Nutrition of Wageningen University (M Engberink).

Donor motivation

Principal investigator
Tini Hoekstra PhD Dept Research and Education South East Region (t.hoekstra@sanquin.nl)

In the past decade the number of blood donors declined steadily in the Netherlands; especially young adults are not well represented in the donor file. Recruitment strategies are the most likely to be successful when they are based on a theory-based understanding of the cognitions, attitudes and beliefs about blood donation and the reasons for non-donation. It is thus essential to understand the cognitions, attitudes and beliefs about blood donation and the reasons for non-donation. A questionnaire was designed based on the Theory of Planned Behavior which includes measures of attitude, social influence, self-efficacy, beliefs, importance and anticipated emotions. The questionnaire was filled in by 311 eligible students. More than 60% indicated they had never seriously thought about becoming a blood donor. The study population was divided into two groups: high and low intenders (i.e. based on their intention to become a blood donor). Both groups acknowledge the same positive aspects of blood donation, but low intenders think donating blood is more uncomfortable, more annoying and more frightening. Both high and low intenders think alike regarding the importance of blood donation. Future research will be performed to study which recruitment strategy will be most effective.

Quality assessment and improvement program

Principal investigator
Ton (A)PM Los Dept Research and Education North East Region (a.los@sanquin.nl)

In 2003 the Quality Assessment and improvement program was continued.
The assessment objective is to ensure that: (a) the interaction between blood bank (personnel) and customers (donors, hospital laboratories is positive, and (b) it promotes good customer service. Additional internal audits between blood bank departments and personnel (communication) are subject of research.

In 2003 the donor satisfaction survey was completed and policy measures taken (regional workgroups) on basis of the results. A communication survey was carried out under the blood bank personnel and results yield the installation of a communication monitoring workgroup.

On behalf of the Board of Directors of Sanquin a national study was carried out under the hospital laboratories focusing on the interaction between the blood bank

department Quality Control, Production Storage and Distribution and Hospital Laboratories.
Also for Sanquin Corporate Staff a national study was carried out under a representative sample from the Dutch female donor population on pregnancy history (relating to TRALI).
Further advisory activities (consultancy) were carried out related to national studies on donor satisfaction, deferred donors and possiblities of using NIPO computer and telephone databases for further donor studies in the future.

Research departments

Department of Clinical Viro-Immunology, Sanquin Research

Department of Experimental Immunohematology, Sanquin Research

Department of Immunopathology, Sanquin Research

Department of Plasma Proteins, Sanquin Research

Department of Transfusion Technology, Sanquin Research

Sanquin Blood Bank North East Region, Department of Research and Education

Sanquin Blood Bank North West Region, Department of Research and Education

Sanquin Blood Bank South East Region, Department of Research and Education

Sanquin Blood Bank South West Region, Department of Research and Education

Department of Clinical Viro-Immunology

Sanquin Research

Address

Sanquin Research
Department of Clinical
Viro-Immunology
Plesmanlaan 125
P.O. Box 9190
NL-1006 AD Amsterdam
The Netherlands
T +31 20 512 33 17
F +31 20 512 33 10
g.damhuis@sanquin.nl
www.cvi.sanquin.nl

Academic staff

J Schuitemaker PhD (head)
Prof F Miedema PhD
NA Tesselaar PhD
D van Baarle PhD
FAC van Engelenburg PhD
*JWAM van Oers PhD**

Postdoctoral fellows

J Borghans PhD
(NWO fellow)
NA Kootstra PhD
(NWO fellow)
*I van den Nieuwenhof PhD**
A van Leeuwen-Gorter

PhD-students

C Bronke (AMC)
I den Braber
CA Jansen
N Kloosterboer
FA Koning
M Navis
E Quakkelaar
E Piriou
E Stalmeijer-Schmidt
N Vrisekoop

Technical staff

S Alders
C Beugeling
BDM Boeser-Nunnink
A Boots
N Brinkhuis
B de Boer
*S de Haan**
SJ Dekker
L Dekker
E Gijsen
A Harskamp-Holwerda
B Hooibrink
C Koevoets
HGH Korsten
M Mangas-Ruiz
A Meinesz
CW Mollenaar
E Mul
NM Nanlohy
J Ottenhoff-Mollenaar
SA Otto
V Purwaha
R Ran
H Smit-Rietkerk
FG Terpstra
F van Alphen
AE van den Blink
TJK van der Vorst
K van Dort
S van Miltenburg
AC van Nuenen
GMW van Schijndel
P van Swieten
JA Velstra-Visser
M Westerlaken
N Willemse
CA Witte

Guest

A Tsegaye (Ethiopia)

Undergraduate students

I Bergval
T Berendsen
B Everts
M Navis
I Rood
A Sanz Sanz

Secretary

GSM Damhuis

* *The research group on TSE's moved to the Dept of Immunopathology* *(page 82)*

Research lines

Department of Experimental Immunohematology

Sanquin Research

Address

Sanquin Research
Department of Experimental
Immunohemaotology
Plesmanlaan 125
P.O. Box 9190
NL-1006 AD Amsterdam
The Netherlands
T +31 20 512 33 77
F +31 20 512 34 74
a.engels@sanquin.nl
www.ihe.sanuin.nl

Academic staff

Prof D Roos PhD (head)
M de Haas MD PhD
PL Hordijk PhD
TW Kuijpers MD PhD (AMC)
CE van der Schoot MD PhD
C Voermans PhD
RS Weening MD PhD
JJ Zwaginga MD PhD

Postdoc fellows

S Beiboer PhD
R van Bruggen (AMC)
KM Dolman MD PhD (AMC)
M Fernandez PhD
N Kamerbeek
WA Noort PhD
PB van Hennik PhD
C van Moorsel PhD

PhD students

M Biezeveld MD (AMC)
N Brouwer (AMC)
S Dohmen MD
MB Hemker MD
J Koelewijn
M Lorenowicz
NA Maianski MD
P Martins da Costa
N Stapleton
GHM Tax (Blood Bank South West Region)
JP ten Klooster
M Tijssen
R van Beem
JD van Buul
N van den Berk
J van Gils

Technical staff

A Ait Soussan
EC Anthony
S Basten
S Bruijns
GMA Cheroutre
M de Boer
A de Vries-van Rossen
R Dee
J Geissler (AMC)
C Homburg (50%)
D van Iersel
J Janssen
IW de Jong
M Kleijer
FPJ Mul
R Siemons
ATJ Tool
M Valk
OJMH Verhagen
A Vos
T Wieringa
R Wijngaarden-du Bois
A Zadurian
L Zappeij
D Zweers
R van Zwieten

Undergraduate students

J Aker
B Kalverda
T Sijswerda

Guests

L Blink PhD (University of Melbourne, Australia)
M Bruin MD (Wilhelmina Childrens Hospital, Utrecht)
V de Haas MD PhD (AMC)
R Endeman MD (Antonius Hospital, Nieuwegein)
Xu Qun MD (Jinan, China, NUFFIC fellow)

Technical trainees

G El Bouchabi
N Buijsman
I Franke
M Hiemstra
S Meisner
A Tol

Secretary

A Engels

Research lines

Department of Immunopathology

Sanquin Research

Address

Sanquin Research
Department of
Immunopathology
Plesmanlaan 125
P.O. Box 9190
NL-1006 AD Amsterdam
The Netherlands
T +31 20 512 31 71
F +31 20 512 31 70
f.muntar@sanquin.nl
www.ip.sanquin.nl

Academic Staff

Prof CE Hack MD PhD (head)
Prof RC Aalberse PhD
Prof LA Aarden PhD
D Hamann PhD
PW Modderman PhD
SM van Ham PhD
R van Ree PhD

Postdoc fellows

GAC Bos
E de Bruin PhD
D Dekkers PhD
R Diemel PhD
M Strik PhD
A ten Brinke PhD
*JWAM van Oers PhD**
*I van den Nieuwenhof PhD**
M van der Neut Kolfschoten PhD
L Zuidmeer PhD

PhD-students

J Akkerdaas
C Ciurana
S de Lathouder
N Diaz Padilla
A Familian
R Kikkert
F McGrath
Y Souwer
E Staal-van Oort
E van Mirre
D Wouters

Technical staff

M Aalbers
R Bosch
B Bottelier
MC Brouwer
I Bulder
D Dam
ER de Groot
S de Haan
P de Heer
N Derksen
MC Dieker
AJM Eerenberg-Belmer
MHL Hart
T Jorritsma
M Karsten
Y Karuntu
H Klaasse-Bos
MJ Kramer
Y Lubbers
R Manoe
M Mulder
S Notten
E Pastoors
HJAM Rensink
D Roem-Haagsma
S Solati
AR van der Horst
T van de Vorst
J van Leeuwen
WA van Leeuwen
G van Mierlo
E Vermeulen
S Versteeg
AM Wolbink-Kamp

Undergraduate students

S Absalah
T Berendsen
G Bertrand
J Cornelissen
I Rood
L van der Zwan
S van Leeuwen

Secretaries

F Muntar
S Sebelon

* *The research group on TSE's joined the department from the Dept Clinical Viro-Immunology*

Research lines

Department of Plasma Proteins

Sanquin Research

Address

Sanquin Research
Department of Plasma
Proteins
Plesmanlaan 125
P.O. Box 9190
NL-1006 AD Amsterdam
The Netherlands
T +31 20 512 31 51
F +31 20 512 36 80
m.vergeer@sanquin.nl
www.pe.sanquin.nl

Academic staff

Prof K Mertens PhD (head)
JA van Mourik PhD
JJ Voorberg PhD

Postdoc fellows

AN Bovenschen PhD
AB Meijer PhD

PhD students

R Bierings
MHA Bos
C Fribourg
V Limburg
B Luken
MJ Hollestelle
J Rohlena
MG Rondaij
M van den Biggelaar
PMW van Helden

Technical staff

RC Boertjes
MG Boon-Spijker
Stempvoort
HJM Brinkman
PPFM Clijsters
K Gijzen
JG Hoogenboezem
PHP Kaijen
JW.M Klein Gebbinck
E Sellink
EAM Turenhout
G van den Brink-van MG
C van der Zwaan
Zuurveld

Secretary

P Hiemstra
MJ Vergeer

Research lines

Department of Transfusion Technology

Sanquin Research

Address

Sanquin Research
Department of Transfusion Technology
Plesmanlaan 125
P.O. Box 9190
NL-1006 AD Amsterdam
The Netherlands
T +31 20 512 33 17
F +31 20 512 33 10
w.winkel@sanquin.nl
www.tt.sanquin.nl

Academic staff

AJ Verhoeven PhD (head)
D de Korte PhD
J Lagerberg PhD

Technical staff

DWC Dekkers
CWN Gouwerok
M Kleine-Go
R Truijens-de Lange
VWK Paeper
JA van Rutten
PM Stokman-Hilarius
AJ van Caldenhove
ChrEM van Rossum
R Verhaar
RC Vlaar

Secretary

W Winkel

Research lines

Sanquin Blood Bank North East Region

Department of Research and Education

Address

Sanquin Blood Bank
North East Region
Department of Research and Education
Prof. Rankestraat 42-44
P.O. Box 1191
NL-9701 BD Groningen
The Netherlands
T +31 50 369 55 55
F +31 50 361 90 39
c.vandelden@sanquin.nl
www.research.sanquin.nl

Academic staff

AL Drayer PhD
MK Elias MD PhD
APM Los
CJ van Delden PhD (head)

Research lines

Sanquin Blood Bank North West Region

Department of Research and Education

Address

Sanquin Blood Bank
North West Region
Department of Research and Education
Plesmanlaan 125
P.O. Box 9137
NL-1006 AC Amsterdam
The Netherlands
T +31 20 512 38 60
F +31 20 617 80 80
d.heidsieck@sanquin.nl
www.research.sanquin.nl

Academic staff

RNI Pietersz, MD PhD (head)
HW Reesink, MD PhD
J Jongerius PhD
WM Smid MD PhD
PJM van den Burg MD PhD
PF van der Meer PhD
J Visser
H Vrielink MD PhD

PhD students

MJ Dijkstra-Tiekstra
T Mohammadi
B Alamdary Badlou

Technicians

M Eijzenga-Demmendal
LAE Liefting
M Habibuw
L Scholtalbers

Secretary

D Heidsieck

Research lines

Sanquin Blood Bank South East Region

Department of Research and Education

Address

Sanquin Blood Bank South East Region
Department of Research and Education
Joseph Bechlaan 113
NL-6229 GR Maastricht
The Netherlands
T +31 43 387 14 56
F +31 43 387 77 80
e.rombout@sanquin.nl
www.research.sanquin.nl

Location Nijmegen:
Van Leeuwenhoeklaan 4
NL-6525 HK Nijmegen
The Netherlands
T +31 24 361 65 82

Academic Staff

E Rombout-Sestrienkova MD (head)
J Curvers PhD
W de Kort MD PhD
T Hoekstra PhD

PhD students

M Engberink
S Cauwenberghs
K.Lemmens
M Luten

Technical staff

J Heeremans
J Klaarenbeek
B Roerdinkholder-Stoelwinder
C Verhagen

Support staff

H Bos PhD
C van Buul PhD
P van Noord MD PhD
J van Wersch PhD
J Wollersheim MD

Undergraduate students

E Theunissen
P van den Bergh

Secretary

E Francken

Research lines

Sanquin Blood Bank South West Region

Department of Research and Education

Address

Sanquin Bloodbank South West Region
Department of Research and Education
Plesmanlaan 1a
NL-2333 BZ Leiden
The Netherlands
T +31 71 568 50 53
F +31 71 568 51 91
anneke.brand@bloodrtd.nl
www.research.sanquin.nl

Location Rotterdam
Wytemaweg 10
NL-3015 CN Rotterdam
T +31 10 463 06 30
F +31 10 463 06 40

Location Dordrecht
Albert Schweizerplaats 5
NL-3318 AS Dordrecht
T +31 78 652 22 22
F +31 78 617 11 00

Academic staff

Prof A Brand MD PhD (head)
Y Hensbergen PhD
PA Maaskant-van Wijk PhD
J Scharenberg PhD
LMG van de Watering MD PhD
JA van Hilten PhD (Sanquin Corporate Staff)
Prof DJ van Rhenen MD PhD

PhD students

JM van Beckhoven
MY Bilgin
MB Hemker
AJG Jansen
F Schipper
C So-Osman MD
GHM Tax (Sanquin Research)
LL Trannoy
MM Waanders

Technical staff

GMAF Cheroutre MC
L Douglas-Berger
J Drunen
M Feskens
A Honohan
K Koekkoek
P de Koning Gans
D Loomans
F Barjiji
J Lorinser
M Slot
H van Vliet
J van Wintershoven

Undergraduate students

M Jansen

Secretary

HMG Harens

Research lines

Product Development Departments

Product and process development, Sanquin Plasma Products

CAF-DCF Brussels, Belgium

Medical Department, Sanquin Plasma Products

Business Unit Reagents, Sanquin Research

Product and process development

Sanquin Plasma Products

Academic staff

AHL Koenderman PhD (head)
M Kleijn PhD

Technical staff

J Bloem
JM Breen
GJ Derksen
HGJ ter Hart
IPP Prins-de Nijs
SNM van 't Schip
N Willem

Secretary

J de Witte-D'Souza

Address

Sanquin Plasma Products
Department of product development
Plesmanlaan 125
P.O. Box 9190
NL-1006 AD Amsterdam
T +31 20 512 39 35
F +31 20 512 36 10
a.koenderman@sanquin.nl
www.sanquin.nl

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)

In 2003, product development focused on the liquid intravenous immunoglobulin project, in which Sanquin Plasma Products is co-operating with the Finnish Red Cross Blood Transfusion Service in Helsinki.

The registration dossier was compiled and the registration application for the new product (Nanogam) was filed in Finland in October 2003. As this product will be manufactured by Sanquin as well, three large scale batches were manufactured in Sanquin's manufacturing facility with the new process installation and the product obtained was used to prove equivalency to Nanogam. A project to characterize liquid immunoglobulin products, including the new Nanogam product, was started in close collaboration with Sanquin Research.

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

A project was started 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. Feasibility on lab scale was shown and experiments on larger scale were started. The next step will be the performance of robustness studies of the virus reducing capacity of this new step.

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

In collaboration with Laboratoire Français du Fractionnement et des Biotechnologies (LFB) in Les Ulis, France, a project was started to implement a 15 nm Planova filtration step in the manufacturing process of Kaskadil to obtain a product comparable to

Sanquin's current product Cofact®, a prothrombin complex concentrate. Feasibility on production scale was shown and the next step will be the manufacturing of product for shelf-life studies, virus validation studies and clinical studies.

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

This 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. Approval to perform a proof-of-principal studies in a limited number of AIDS patients was obtained and the studies are scheduled to take place in 2004. This development project is executed in cooperation with the University Center for Pharmacy, State University of Groningen and the International Antiviral Therapy Evaluation Center (IATEC) of the Academic Medical Center of Amsterdam.

CAF-DCF Brussels, Belgium

Central Fractionation Department of the Belgian Red Cross

Academic staff

PFW Strengers MD (Director Medical Affairs and Product Development)
R Laub PhD (head)
M Di Giambattista Ig (supervisor)

Technical staff

Th Branckaert
V Hougardy
V Labrune
F Reyé
B Vandenborre

Secretary

B Kips

Address

CAF-DCF
De Tyraslaan 109
B 1120 Brussel
T +32 2 264 64 90
F +32 2 262 27 31
ruth.laub@caf-dcf.redcross.be

The objectives of the Department of Product Support (former R&D) are to ensure the quality and safety of the plasma derivatives, manufactured at CAF-DCF. Main concerns are to develop new tools for use at two levels: reducing the bio-burden of pathogens in the starting manufacture plasma pools to a level that can be handled by inactivation/removal methods and complementary inactivation technologies including UV technology, active against the largest range of blood-borne viruses. State-of-the-art methodologies for characterizing plasma proteins, both immunological (e.g. epitope identification) and biochemical, are developed and exploited in industrial applications.

UV technology as a pathogen inactivation tool

For various reasons related to public health, it is increasingly clear that new virus elimination or inactivation methods must be developed. Some viruses, and notably parvoviruses and erythrovirus (formerly Parvovirus) B19, are particularly resistant to current methods. We have studied the inactivation of viruses used to spike different plasma protein matrixes such as FVIII, immunoglobulins, fibrinogen, and albumin. Under conditions preserving protein activity, we have compared the efficacy of different methods against the following viruses: murine parvovirus MVMp, human B19, the encephalomyocarditis virus (EMC, a picornavirus used as a model for hepatitis A virus), and bovine herpes virus type 1 (BHV, a model for enveloped viruses like hepatitis B virus). We have shown that dynamic continuous-flow UVC irradiation is very effective, particularly against resistant pathogens (e.g. Parvoviruses and bacteria) without addition of any photosensitizers or free-radical scavengers. In our irradiator, both DNA and RNA viruses are inactivated when the fluence exceeds 240 J/m^2. At dosages equal to or exceeding 480 J/m^2, B19 multiplication (measured by a sensitive NAT) in the KU812F cell model is markedly reduced and that the rare progeny of the irradiated viruses are no longer infectious in a second-round infection. Lab-scale and production-scale UV inactivation and disinfection systems have been developed. GMP compliant, fully validated, highly compact and low priced, these systems are easy to implement in any biotechnological manufacturing process. Further modification makes possible to use our irradiation system to study the efficacy of different UV wavelengths such as UVA in the presence or absence of photoreactive compounds.

New virus inactivation technologies such as UV treatment will extend blood product safety as regards a wider range of pathogens.

Key publication

Caillet-Fauquet PC, Di Giambattista M, Draps ML, Sandras F, Branckaert Th,de Launoit Y and Laub R. Continuous-flow UVC irradiation: a new, effective, protein-activity-preserving system for inactivating bacteria and viruses, including erythrovirus B19. J Virol Methods 2004; 118(2):131-9.

High-titer B19-DNA screening by NAT of 852,853 donations collected in Belgium in 2002–2003 (different pooling strategies were used)

Major efforts are being made to eliminate erythrovirus (formerly Parvovirus) B19 (B19V) before its inclusion in the manufacture pool. Human B19 is regarded as a potential risk for patients treated with plasma derivatives. Since October 2002, samples of all donations provided by the Belgian Blood Transfusion Centers have been pooled in sub-pools of 96 donations (midi-pools) and then 576 donations (maxi-pools). As no single sampling system prevails in Belgium, two different pooling strategies were developed depending of the sample format: a sample of individual donations in multiwell plates (strategy A) and tubes containing 8 samples issued from 8 donations (strategy B). When a maxi-pool (576 donations) was found B19-DNA positive by PCR, the maxi-pool was resolved to midi-pools for further B19-DNA detection. Different intersecting smaller subpools were constructed and analyzed so as to identify the contaminated donation in the multiwell plates or in the tube containing 8 donation samples. The low-cost in-house PCR test (95% sensitivity: 4800 IU/ml assessed by different methods and confirmed by real-time PCR) contains an internal control and an in-house working standard (low and high viral concentrations) and was validated according to the specific B19 OMCL guidelines and in inter-laboratory studies.
Out of a total of 852,853 donations, 1543 maxi-pools (578 donations) were constructed and analyzed. 81 maxi-pools were found positive. A total of 159 donations were identified as positive, showing a prevalence for B19 of 1.9/10,000 donations. Most B19-DNA-positive donations were found in the period February-May 2003.
The results show that the use of intersecting pools makes it possible to identify the positive donation or pool of 8 donations. Pooling individual donations is slightly more time consuming but allows identification of the positive donation unit in one run, focusing only on samples (strategy A). The identity of the contaminated donation was always confirmed. To identify the positive donation in pools of 8 samples

(strategy B), the 'pig tails' of the 8 donation bags must be collected and analyzed. The results also show that the fractionation process has sufficient virus inactivation/removal capacity for the residual B19 titer in plasma-pools.

The studies described here were performed in collaboration with Dr I Thomas (Institut Scientifique de Santé Publique – Louis Pasteur, Brussels), Dr PC Caillet-Fauquet and Prof Y de Launoit (ULB – Laboratoire de Virologie Moléculaire, Brussels). Collaborations are running also with L Craciun, E Dupont and M Goldman (ULB – Laboratoire d'Immunologie Expérimentale, Brussels).

Key publication

Thomas I, Di Giambattista M, Gerard C, Mathys E, Hougardy V, Latour B, Branckaert T, Laub R. Prevalence of human erythrovirus B19 DNA in healthy Belgian blood donors and correlation with specific antibodies against structural and non-structural viral proteins. Vox Sang 2003; 84(4):300-7.

Medical Department

Sanquin Plasma Products

Staff Medical Department

PFW Strengers MD (Director Medical Affairs and Product Development)

Medical Advisors

JJ Marcar MD
TJ Schouten MD PhD

Clinical Research Associates

JC Drenth, MSc
A Faber
I Kleine Budde, PhD
PJM Vossebeld, PhD

Secretary

P Niekoop-Snijders

Address

Sanquin Plasma Products
Medical Department
Plesmanlaan 125
P.O. Box 9190
NL-1006 AD Amsterdam
The Netherlands
T +31 20 512 32 39
F +31 20 512 37 94
p.niekoop@sanquin.nl

The main objective of the Medical Department is developing and performing clinical trials with plasma products in order to obtain marketing authorization, new indication(s) or new therapeutic modalities for new or authorized plasma products.

These clinical studies are designed in close collaboration with investigators in the Netherlands and abroad, in particular with the Inter-University Working Party on the Study of Immune Deficiencies and the Hemophilia Treatment Centers. The department is involved in pharmacovigilance: a system of activities to monitor safety of medicinal products in regular medical care in order to prevent the occurrence or recurrence of adverse drug reactions. The system consists of passive pharmacovigilance based on received reports, and active pharmacovigilance by performing post marketing surveillance clinical studies in ad random patient groups. The data of pharmacovigilance are presented in Periodic Safety Update Reports (PSURs) and in scientific papers. The Drug Safety Officer has prepared a safety report for PCC-SD, and a PSUR for intramusculair immunoglobuline for a five years review period, which was required for re-registration. Regular PSURs were submitted for Nonafact®, twice, and for Ivegam®.

The Medical Department provides medical information and advice to physicians, nurses and pharmacists on the usage (e.g. dosage, indications, administration) of plasma products in order to safeguard the clinical use of plasma products. Oral presentations are given on the clinical use and indications of these products. In the provision of specific source plasma from plasmapheresis donors for the fractionation of anti-rhesus (D) immunoglobulin, the Medical Department assists in the recruitment of new plasmapheresis donors and performs the selection of specific units of erythrocytes for immunisation, to be used by Sanquin Blood Banks.

Clinical trials ongoing in 2003

Nonafact

The objectives of the study 'Post marketing study in hemophilia B patients using Nonafact® 100 IU/ml powder and solvent for solution for injection (human coagula-

IvIG showed excellent efficacy and excellent safety during treatment of 18 patients with primary immune deficiency.

tion factor IX) (human plasma derived factor IX product, liophilized' are to study the safety of treatment with Nonafact® in every aspect of regular patient treatment. This ongoing study was started in four Hemophilia Treatment Centers in the Netherlands.

IVIG-L

A recently developed liquid intravenous immunoglobulin product, IVIG-L, has passed several clinical trials. The final report of the clinical study 'Kinetics, efficacy and safety of IVIG-L (human normal immunoglobulin for intravenous use) in patients with hypogammaglobulinemia' showed the excellent efficacy, based on the number of infections and plasma trough level of IgG, and excellent safety during treatment of 18 patients with primary immune deficiency.

PPSB-SD

The 'Study on the efficacy of PPSB Solvent Detergent® and VP-VI in patients using oral anticoagulant therapy and undergoing acute cardiac surgery with a cardiopulmonary by-pass' was set up in collaboration with the Medical Department of CAF-DCF cvba, the alliance partner of Sanquin, The objective of the study is comparing the efficacy of treatment with PPSB Solvent Detergent with the efficacy of the standard treatment with SD treated Fresh Frozen Plasma (FFP) in 40 patients. The study is being carried out at the Academic Hospital of the Catholic University 'Gasthuisberg', Leuven, Belgium.

MBL

A phase II study with MBL (Mannan Binding Lectin), a product from Staten Serum Institute (SSI), Copenhagen, Denmark, was set up to investigate the pharmacokinetic profile and the clinical and biological effects of MBL replacement therapy in 12 MBL-deficient children during chemotherapy-induced neutropenia. The study protocol 'Phase II study on Mannan Binding Lectin (MBL) substitution in MBL-deficient children with chemotherapy-induced neutropenia' was approved by the Ethical Advisory Board of Academic Medical Center in Amsterdam.

C1-esterase inhibitor

To further optimize the viral safety of Cetor®, a 15 nm-nanofiltration step will be incorporated in the production process of Cetor®. A study to demonstrate (pharmacokinetic) equivalence between the current Cetor® product and nanofiltrated Cetor® is in preparation.

Business Unit Reagents

Sanquin Research

Staff

R Melsert PhD (head)
A Blom-Bijvoet PhD
A de Bot
L de Jong
V Purwaha
R Reijneke
H Schoenmakers
P Stam
P Stekkinger
H te Velthuis PhD
E van der Donk
W van Esch PhD
H van Lierop
A Verkamman
L Visser
J Voorn

Address

Sanquin Research
Business Unit Reagents
Plesmanlaan 125
P.O. Box 9190
NL-1006 AD Amsterdam
The Netherlands
T +31 20 512 35 53
F +31 20 512 34 31
r.melsert@sanquin.nl
www.reagents.sanquin.nl

Sanquin Reagents, within the organisation known as the BUR (Business Unit Reagents), is as 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.

The European Union's IVD Directive

As a producer of reagents used for *in vitro* diagnostics (IVD), Sanquin Reagents had a tough deadline of December 7, 2003 to comply with the new CE-IVD Directive 98/79/EC. From the beginning of 2003 onwards three BUR project teams performed the necessary work, for the various IVD productgroups (human blood grouping reagents, human IgG subclass reagents, and human leukocyte differentiation antigens or HLDA CDs). At the end of November this work was successfully finished, and all IVD products produced by Sanquin Reagents were CE-marked.

Business unit strategy

During the second half of 2003, the BUR management team performed SWOT analyses of the business unit and it's product lines. These analyses were the basis for defining the long term business unit strategy, and for updating the current project portfolio.

R&D project teams

In 2003 the BUR research and product development activities focused on several key projects. Red cells from Sanquin donors were processed to be used in cell panels. With respect to Cellbind, adaptation of the column interior and further development of semi- and fully automated user instruments were undertaken. New human IgG subclass reagents were developed. Kits for human MBL (Mannan Binding Lectin) and work was done on ELISPOT kits and antibody pair.

Focus was put on recombinant antibody technology and MHC tetramer technology. With respect to multi-analyte detection systems, effort was put in the Luminex project and the blood group DNA chip in collaboration with Sanquin Research and Sanquin Diagnostic Services.

New products

The following new products were commercially introduced: Latex based human IgG subclass reagents, various ELISPOT reagents, e.g. the human granzyme B ELISPOT kit, and an ELISPOT scanner for analysis of ELISPOT filters (AELVIS). Also monoclonal antibody based reagents for blood group typing, and 0.8% red cell panels for column techniques as well as human MHC class I tetramers were introduced.
Furthermore several MACS products from Miltenyi were introduced in the portfolio.

Services Departments

Sanquin Pharmaceutical Services, Sanquin Research

Virus Safety Services, Sanquin Research

Sanquin Consulting Services

Sanquin Pharmaceutical Services

Sanquin Research

Academic Staff

PC van Mourik (head)
EJM Al PhD
NJJ Dekker
A de Jonge
HMG Sillekens

Technical staff

M Boer
MWG Botter-Lavrijsen
M Breman
R Bron
A Griffioen-van der Zwet
T Lagendijk-Rogic
M Lynch
MLE Peekel
B van Druten-Woudenberg
S van Houten
BJ Verdonk

Support staff

J Botterman
KHM Klemann

Address

Sanquin Research
Sanquin Pharmaceutical Services
Plesmanlaan 125
P.O. Box 9190
NL-1006 AD Amsterdam
The Netherlands
T +31 20 512 31 93

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. Moreover, process validation studies in order to demonstrate the reduction of (model) viruses or DNA during purification as well as the validation of client dedicated assays are part of their dedicated activities.

Virus Safety Services

Sanquin Research

Academic staff

FG Terpstra (laboratory head)
FAC van Engelenburg PhD

Technical staff

AGC Boots
LM Bos
FHM Brinkhuis
E Gijsen
HGH Korsten
AE van den Blink
CA Witte

Address

Sanquin Research
Virus Safety Services
Plesmanlaan 125
P.O. Box 9190
NL-1006 AD Amsterdam
The Netherlands
T +31 20 512 35 94
F +31 20 512 33 10
f.terpstra@sanquin.nl
www.vss.sanquin.nl

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 organisation.

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).

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
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
VSV (Vesicular stomatitis virus), a general model for lipid enveloped RNA viruses

Sanquin Consulting Services

Staff

Prof ChT Smit Sibinga MD PhD (head)
C Kooistra
APM Los
WPA van der Tuuk Adriani MD PhD

Address

Sanquin Consulting Services
AZG Triade 24, r 210
P.O. Box 11085
NL-9700 CB Groningen
T +31 50 361 00 62
F +31 50 361 90 39
scs@sanquin.nl
www.scs.sanquin.nl

Sanquin Consulting Services is an internationally facilitating unit of the Sanquin Blood Supply Foundation in the Netherlands providing guidance 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 programs 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 organisation.

Organisation and structure

A study provided a basic model design of a structure that could accommodate the three different though interrelated aspects

– Sanquin Consulting Services for the field work (projects and consultations),
– WHO Collaborating Center for the vocational training, and
– Academic Institute for International Development of Transfusion Medicine (IDTM)

accommodating academic education (postgraduate Master curriculum) and health sciences research focused on transfusion medicine.
Based on the report the services started to build up a quality system and management based on ISO 9000/2000 principles with the goal to accomplish inspection and certification by the end of 2004.

The services started to build up consulting capacity from the ranks of Sanquin through focused information and a call for participation: some 40 Sanquin employees from the different divisions and the Corporate Staff showed interest and are registered.

Public relations and acquisition

Development of visibility and public relations, both within the organization and the country as well as internationally, was realized through:

– A web page at the Sanquin website.
– An active project acquisition program to safeguard sustainability for the future.

– The emphasis is on the managerial aspects through guidance support and advice how to create and implement nationally supported organisational structures and comprehensive quality systems and management.

AABB consulting services division

By the end of 2003 the agreement with AABB to share the leadership of Sanquin Consulting Services (SCS) and Consulting Services Division (CSD) was continued.

WHO relationship

To extend the collaborating activities and support to the entire Sanquin organization, Sanquin Blood Supply Foundation has send WHO a proposal of broadening existing collaborative activities.

Academic institute for international development of transfusion medicine

Development of the postgraduate Master curriculum and course was started. The design chosen is modular with an extensive 12 months e-learning part (9 modules) allowing fellows to complete within their own environment and a 6 months (4 modules) tutorial and practical exposure part to be completed in the Netherlands. The graduation will be based on a thesis project (last module). Finally the University of Groningen Faculty of Medical Sciences will formally provide the Master degree and diploma.

Activities and projects

Sanquin Consulting Services, the WHO Collaborating Center and the Academic Institute IDTM have been involved in a variety of activities and projects:
For more details, please contact us for the annual report of SCS, IDTM.

Sponsors

Various organisations, 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 AMC-Sanquin Landsteiner Laboratory, housed in Sanquin's premises in Amsterdam.

National and international Research Councils, governmental organisations

CvZ-College van Zorgverzekeraars

Deutsche Forschungsgemeinschaft

Dutch Medical Research Council (ZON/MW)

European Commission

Ministry of Economic Affairs (WBSO)

Ministry of Public Health, Welfare and Sport

Municipal Health Services Amsterdam (GG&GD)

National AIDS Therapy Evaluation Center

Netherlands Organisation for Scientific Research (NWO)

Royal Netherlands Academy of Arts and Sciences

SENTER/Novem

Charities and private funding organisations

Chronic Gronulomatosis Disease Trust

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)

Leiden University Fund

National Foundation for Rheumatism

Nefkens Foundation

Netherlands Asthma Foundation

Platform Alternatieve Dierproeven

Princess Beatrix Foundation

Stichting Fondsenwervingsacties Volksgezondheid

Tekke Huizinga Foundation

Contract and co-development partners

Academic Hospital, University of Maastricht

Academic Medical Center, University of Amsterdam

Adenbrooks Hospital

American Red Cross

Amcell Corp.

ASAHI-Medical Co

Astra-Tech

A-Viral ASA

Baxter BioScience

Baxter Health Care

Baxter Oncology

Berna Biotech

BioMérieux Nederland

Bioplex

Biosafe

Biotest Pharma GmbH

Boehringer Ingelheim Pharmaceuticals Inc.
Cardiovascular Research Institute Maastricht (CARIM)
Cellgenix
Centeon
Cerus Corp
Chiron corporation
Copenhagen University
Diaclone
Diagnos Biochemical Cattle Management
DSM Biologics
Finnish Red Cross
Fresenius HemoCare
Fuji
Gambro BCT
Genentech
Genmab
GlaxoSmithKline
Guava Techmologies
Haemonetics
HAL/Madaus
Harimex
ILSI
Immulogic
Innogenetics
Jan van Bremen Institute
Kamada
Leiden University Medical Center
Macopharma
Magen David Adom
Microsafe BV
Miltenyi Biotech
Morphosis AG
Natal Bioproducts Institute
Navigant Bonville
Nedalco
NIZO laboratories
OncoMab
Ortho-Clinical Diagnostics
Pharming
PhotoBioChem
ProLacta
Région de Bruxelles-Capitale
RIVM, National Institute for Public Health and the Environment
Roche Diagnostics
Roche Nederland
Seattle Genetics
Schering Corporation
Slotervaart Hospital
Stallergéne
Staten Serum Institute
Surgical Commpany International
Synaps BV
Synco
Universiteit Amsterdam
University Medical Center Utrecht
Van Straten Medical
Vitaleech
Vrije Universiteit Medical Center, Amsterdam
Wageningen University and Research Center
Zentech, s.a.
Zentral Laborator Bern

Publications

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

Papers in international journals

Alphabetically, first author

Akkerdaas JH, Wensing M, Knulst AC, Krebitz M, Breiteneder H, de Vries S, Penninks AH, Aalberse RC, Hefle SL, van Ree R. How accurate and safe is the diagnosis of hazelnut allergy by means of commercial skin prick test reagents? Int Arch Allergy Immunol 2003; 132(2):132-40.

Astermark J, Voorberg J, Lenk H, DiMichele D, Shapiro A, Tjonnfjord G, Berntorp E. Impact of inhibitor epitope profile on the neutralizing effect against plasma-derived and recombinant factor VIII concentrates *in vitro*. Haemophilia 2003; 9(5):567-72.

Badolato R, Notarangelo LD, Plebani A, Roos D. Development of systemic lupus erythematosus in a young child affected with chronic granulomatous disease following withdrawal of treatment with interferon-gamma. Rheumatology (Oxford) 2003; 42(6): 804-5.

Beezhold DH, Hickey VL, Kostyal DA, Puhl H, Zuidmeer L, van Ree R, Sussman GL. Lipid transfer protein from Hevea brasiliensis (Hev b 12), a cross-reactive latex protein. Ann Allergy Asthma Immunol 2003; 90(4):439-45.

Beillard E, Pallisgaard N, van der Velden VHJ, Bi W, Dee R, van der Schoot E, Delabesse E, Macintyre E, Gottardi E, Saglio G, Watzinger F, Lion T, van Dongen JJ, Hokland P, Gabert J. Evaluation of candidate control genes for diagnosis and residual disease detection in leukemic patients using 'real-time' quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR) – a Europe against cancer program. Leukemia 2003; 17(12):2474-86.

Besselink GA, Ebbing IG, Hilarius PM, de Korte D, Verhoeven AJ, Lagerberg JW. Composition of the additive solution affects red blood cell integrity after photodynamic treatment. Vox Sang 2003; 85(3):183-9.

Besselink GA, van Engelenburg FA, Ebbing IG, Hilarius PM, de Korte D, Verhoeven AJ. Additive effects of dipyridamole and Trolox in protecting human red cells during photodynamic treatment. Vox Sang 2003; 85(1):25-30.

Biezeveld MH, Kuipers IM, Geissler J, Lam J, Ottenkamp JJ, Hack CE, Kuijpers TW. Association of mannose-binding lectin genotype with cardiovascular abnormalities in Kawasaki disease. Lancet 2003; 361(9365):1268-70.

Biro E, Sturk-Maquelin KN, Vogel GM, Meuleman DG, Smit MJ, Hack CE, Sturk A, Nieuwland R. Human cell-derived microparticles promote thrombus formation *in vivo* in a tissue factor-dependent manner. J Thromb Haemost 2003; (12):2561-8.

Boer AK, Drayer AL, Vellenga E. cAMP/PKA-mediated regulation of erythropoiesis. Leuk Lymphoma 2003; 44(11):1893-901. Review.

Boer AK, Drayer AL, Vellenga E. Stem cell factor enhances erythropoietin-mediated transactivation of signal transducer and activator of transcription 5 (STAT5) via the PKA/CREB pathway. Exp Hematol 2003; 31(6):512-20.

Bogaards JA, Weverling GJ, Geskus RB, Miedema F, Lange JM, Bossuyt PM, Goudsmit J. Low versus high CD4 cell count as starting point for introduction of antiretroviral treatment in resource-poor settings: a scenario-based analysis. Antivir Ther 2003; 8(1):43-50.

Bos IG, de Bruin EC, Karuntu YA, Modderman PW, Eldering E, Hack CE. Recombinant human C1-inhibitor produced in Pichia pastoris has the same inhibitory capacity as plasma C1-inhibitor. Biochim Biophys Acta 2003; 1648(1-2):75-83.

Bos IG, Lubbers YT, Roem D, Abrahams JP, Hack CE, Eldering E. The functional integrity of the serpin domain of C1-inhibitor depends on the unique N-terminal domain, as revealed by a pathological mutant. J Biol Chem 2003; 278(32):29463-70.

Bos JC, Toorians AW, van Mourik JC, Strack van Schijndel RJ. Emergency resection of an extra-adrenal phaeochromocytoma: wrong or right? A case report and a review of literature. Neth J Med 2003; 61(8):258-65.

Bovenschen N, Boertjes RC, van Stempvoort G, Voorberg J, Lenting PJ, Meijer AB, Mertens K. Low density lipoprotein receptor-related protein and factor IXa share structural requirements for binding to the A3 domain of coagulation factor VIII. J Biol Chem 2003; 278(11):9370-7.

Bovenschen N, Herz J, Grimbergen JM, Lenting PJ, Havekes LM, Mertens K, van Vlijmen BJ. Elevated plasma factor VIII in a mouse model of low-density lipoprotein receptor-related protein deficiency. Blood 2003; 101(10):3933-9.

Brand A. Does blood transfusion increase risk of postoperative complications? Acta Anaesthesiol Belg 2003; 54(4):297.

Branger J, van den Blink B, Weijer S, Gupta A, van Deventer SJH, Hack CE, Peppelenbosch MP, van der Poll T. Inhibition of coagulation, fibrinolysis, and endothelial cell activation by a p38 mitogen-activated protein kinase inhibitor during human endotoxemia. Blood 2003; 101(11):4446-8.

Burritt JB, Foubert TR, Baniulis D, Lord CI, Taylor RM, Mills JS, Baughan TD, Roos D, Parkos CA, Jesaitis AJ. Functional epitope on human neutrophil flavocytochrome b558. J Immunol 2003; 170(12):6082-9.

Cadot P, Kochuyt AM, van Ree R, Ceuppens JL. Oral allergy syndrome to chicory associated with birch pollen allergy. Int Arch Allergy Immunol 2003; 131(1):19-24.

Daniels GL, Cartron JP, Fletcher A, Garratty G, Henry S, Jorgensen J, Judd WJ, Levene C, Lin M, Lomas-Francis C, Moulds JJ, Moulds JM, Moulds M, Overbeeke M, Reid ME, Rouger P, Scott M, Sistonen P, Smart E, Tani Y, Wendel S, Zelinski T. International Society of Blood Transfusion Committee on terminology for red cell surface antigens: Vancouver Report. Vox Sang 2003; 84(3):244-7.

Davis C, Heath A, Best S, Hewlett I, Lelie N, Schuurman R, Holmes H. Calibration of HIV-1 working reagents for nucleic acid amplification techniques against the 1st international standard for HIV-1 RNA. J Virol Methods 2003; 107(1):37-44.

De Jager W, te Velthuis H, Prakken BJ, Kuis W, Rijkers GT. Simultaneous detection of 15 human cytokines in a single sample of stimulated peripheral blood mononuclear cells. Clin Diagn Lab Immunol 2003; 10(1):133-9.

De Kleijn ED, de Groot R, Hack CE, Mulder PG, Engl W, Moritz B, Joosten KF, Hazelzet JA. Activation of protein C following infusion of protein C concentrate in children with severe meningococcal sepsis and purpura fulminans: a randomized, double-blinded, placebo-controlled, dose-finding study. Crit Care Med 2003; 31(6):1839-47.

De Wildt-Eggen J, Gullikson H. *In vivo* and *in vitro* comparison of platelets stored in either synthetic media or plasma. Vox Sang 2003; 84(4):256-64.

Diaz Padilla N, Bleeker WK, Lubbers Y, Rigter GMM, van Mierlo GJ, Daha MR, Hack CE. Rat C-reactive protein activates the autologous complement system. Immunology 2003; 109(4): 564-71.

Dijkstra-Tiekstra MJ, de Korte D, Pietersz RNI, Reesink HW, van der Meer PF, Verhoeven AJ. Comparison of various dimethylsulphoxide-containing solutions for cryopreservation of leucoreduced platelet concentrates. Vox Sang 2003; 85(4): 276-82.

Dijkstra-Tiekstra MJ, Schrijver JG, van der Meer PF, Laport RF, Gratama JW, Levering WH, van Delden CJ, Wildt-Eggen J. Crossover study of three methods for low-level white blood cell counting on two types of flow cytometer. Cytometry 2003; 54B(1):39-45.

Eijkhout HW, van den Broek PJ, van der Meer JW. Substitution therapy in immunodeficient patients with anti-IgA antibodies or severe adverse reactions to previous immunoglobulin therapy. Neth J Med 2003; 61(6):213-7.

Elias M, Choudhury N, Sibinga CT. Cord blood from collection to expansion: feasibility in a regional blood bank. Indian J Pediatr 2003; 70(4):327-36.

Engelfriet CP, Reesink HW, Klein HG, AuBuchon JP, Strauss RG, Krusius T, Maki T, Rebulla P, Hogman CF, Knutson F, Letowska M, Dickmeiss E, Winter M, Henn G, Menichetti E, Mayr WR, Flanagan P, Martin-Vega C, Massuet L, Wendel S, Turek P, Lin CK, Shirato T. The future use of pathogen-inactivated platelet concentrates. Vox Sang 2003; 85(1):54-66.

Engelfriet CP, Reesink HW, Kroll H, Giers G, Bald R, Kanhai H, Kekomaki R, Teramo K, Panzer S, Jilma P, Ulm B, Bock J, Taaning E, Rodeck C, Goldman M, David M, Kaplan C. Prenatal management of alloimmune thrombocytopenia of the fetus. Vox Sang 2003; 84(2):142-9.

Fijnvandraat K, Bril WS, Voorberg J. Immunobiology of inhibitor development in hemophilia A. Semin Thromb Hemost 2003; 29(1):61-8.

Fraaij PL, van Rossum AM, Wolthers KC, Vossen AC, Jeucken YM, Kuijpers JH, van Lochem EG, Roos MT, de Groot R, Hartwig NG. Initation of highly active antiretroviral therapy leads to an HIV-specific immune response in a seronegative infant. AIDS 2003; 17(1):138-40.

Fuhler GM, Drayer AL, Vellenga E. Decreased phosphorylation of protein kinase B and extracellular signal-regulated kinase in neutrophils from patients with myelodysplasia. Blood 2003; 101(3):1172-80.

Fuhler GM, Hooijenga F, Drayer AL, Vellenga E. Reduced expression of flavocytochrome b558, a component of the NADPH oxidase complex, in neutrophils from patients with myelodysplasia. Exp Hematol 2003; 31(9):752-9.

Geels MJ, Cornelissen M, Schuitemaker H, Anderson K, Kwa D, Maas J, Dekker JT, Baan E, Zorgdrager F, van den Burg R, van Beelen M, Lukashov VV, Fu TM, Paxton WA, van der Hoek L, Dubey SA, Shiver JW, Goudsmit J. Identification of sequential viral escape mutants associated with altered T-cell responses in a human immunodeficiency virus type 1-infected individual. J Virol 2003; 77(23):12430-40.

Gerards AH, de Lathouder S, de Groot ER, Dijkmans BA, Aarden LA. Inhibition of cytokine production by methotrexate. Studies in healthy volunteers and patients with rheumatoid arthritis. Rheumatology (Oxford) 2003; 42(10):1189-96.

Geskus RB, Miedema F, Goudsmit J, Reiss P, Schuitemaker H, Coutinho RA. Prediction of residual time to AIDS and death based on markers and cofactors. J Acquir Immune Defic Syndr 2003; 32(5):514-21.

Groeneveld AB, Tacx AN, Bossink AW, van Mierlo GJ, Hack CE. Circulating inflammatory mediators predict shock and mortality in febrile patients with microbial infection. Clin Immunol 2003; 106(2):106-15.

Gulliksson H, AuBuchon JP, Cardigan R, van der Meer PF, Murphy S, Prowse C, Richter E, Ringwald J, Smacchia C, Slichter S, Wildt-Eggen J. Storage of platelets in additive solutions: a multicenter study of the *in vitro* effects of potassium and magnesium. Vox Sang 2003; 85(3):199-205.

Hack CE. Derangements of coagulation and fibrinolysis in infectious diseases. Contrib Microbiol 2003; 10:18-37.

Hardeman MR, Besselink GA, Ebbing I, de Korte D, Ince C, Verhoeven AJ. Laser-assisted optical rotational cell analyzer measurements reveal early changes in human RBC deformability induced by photodynamic treatment. Transfusion 2003; 43(11): 1533-7.

Hazenberg MD, Borghans JAM, de Boer RJ, Miedema F. Thymic output: a bad TREC record. Nat Immunol 2003; 4(2):97-9.

Hazenberg MD, Otto SA, Hamann D, Roos MTL, Schuitemaker H, de Boer RJ, Miedema F. Depletion of naive CD4 T-cells by CXCR4-using HIV-1 variants occurs mainly through increased T-cell death and activation. AIDS 2003; 17(10):1419-24.

Hazenberg MD, Otto SA, van Benthem BHB, Roos MTL, Coutinho RA, Lange JMA, Hamann D, Prins M, Miedema F. Persistent immune activation in HIV-1 infection is associated with progression to AIDS. AIDS 2003; 17(13):1881-8.

Hemker MB, Cheroutre G, van Zwieten R, Maaskant-van Wijk PA, Roos D, Loos JA, van der Schoot CE, von dem Borne AEGK. The Rh complex exports ammonium from human red blood cells. Br J Haematol 2003; 122(2):333-40.

Hermsen CC, Konijnenberg Y, Mulder L, Loe C, van Deuren M, van der Meer JWM, van Mierlo GJ, Eling WM, Hack CE, Sauerwein RW. Circulating concentrations of soluble granzyme A and B increase during natural and experimental Plasmodium falciparum infections. Clin Exp Immunol 2003; 132(3):467-72.

Hesselink DA, Aarden LA, Swaak AJ. Profiles of the acute-phase reactants C-reactive protein and ferritin related to the disease course of patients with systemic lupus erythematosus. Scand J Rheumatol 2003; 32(3):151-5.

Himly M, Jahn-Schmid B, Dedic A, Kelemen P, Wopfner N, Altmann F, van Ree R, Briza P, Richter K, Ebner C, Ferreira F. Art v 1, the major allergen of mugwort pollen, is a modular glycoprotein with a defensin-like and a hydroxyproline-rich domain. FASEB J 2003; 17(1):106-8.

Hordijk P. Endothelial signaling in leukocyte transmigration. Cell Biochem Biophys 2003; 38(3):305-22.

Jansen AJ, Essink-Bot ML, Beckers EA, Hop WC, Schipperus MR, van Rhenen DJ. Quality of life measurement in patients with transfusion-dependent myelodysplastic syndromes. Br J Haematol 2003; 121(2):270-4.

Kassu A, Tsegaye A, Wolday D, Petros B, Aklilu M, Sanders EJ, Fontanet AL, van Baarle D, Hamann D, de Wit TF. Role of incidental and/or cured intestinal parasitic infections on profile of $CD4^+$ and $CD8^+$ T-cell subsets and activation status in HIV-1 infected and uninfected adult Ethiopians. Clin Exp Immunol 2003; 132(1):113-9.

Kerkhof M, Koopman LP, van Strien RT, Wijga A, Smit HA, Aalberse RC, Neijens HJ, Brunekreef B, Postma DS, Gerritsen J. Risk factors for atopic dermatitis in infants at high risk of allergy: the PIAMA study. Clin Exp Allergy 2003; 33(10):1336-41.

Kleine Budde I, Aalberse RC. Histamine-releasing factors, a heterogeneous group of different activities. Clin Exp Allergy 2003; 33(9):1175-82.

Koning FA, Kwa D, Boeser-Nunnink B, Dekker J, Vingerhoed J, Hiemstra H, Schuitemaker H. Decreasing sensitivity to RANTES (regulated on activation, normally T-cell-expressed and -secreted) neutralization of CC chemokine receptor 5-using, non-syncytium-inducing virus variants in the course of human immunodeficiency virus type 1 infection. J Infect Dis 2003; 188(6):864-72.

Koraka P, Murgue B, Deparis X, Setiati TE, Suharti C, van Gorp EC, Hack CE, Osterhaus AD, Groen J. Elevated levels of total and dengue virus-specific immunoglobulin E in patients with varying disease severity. J Med Virol 2003; 70(1):91-8.

Krijnen PA, Meischl C, Hack CE, Meijer CJ, Visser CA, Roos D, Niessen HW. Increased Nox2 expression in human cardiomyocytes after acute myocardial infarction. J Clin Pathol 2003; 56(3):194-9.

Krijnen PA, Meischl C, Visser CA, Hack CE, Niessen HW, Roos D. NAD(P)H oxidase in the failing human heart. J Am Coll Cardiol 2003; 42(12):2170-1.

Kuijpers TW, Biezeveld M, Achterhuis A, Kuipers I, Lam J, Hack CE, Becker AE, van der Wal AC. Longstanding obliterative panarteritis in Kawasaki disease: lack of cyclosporin A effect. Pediatrics 2003; 112(4):986-92.

Kuijpers TW, Maianski NA, Tool AT, Smit GP, Rake JP, Roos D, Visser G. Apoptotic neutrophils in the circulation of patients with glycogen storage disease type 1b (GSD1b). Blood 2003; 101(12): 5021-4.

Kuijpers TW, Ridanpaa M, Peters M, de Boer I, Vossen JM, Pals ST, Kaitila I, Hennekam RC. Short-limbed dwarfism with bowing, combined immune deficiency, and late onset aplastic anaemia caused by novel mutations in the RMPR gene. J Med Genet 2003; 40(10):761-6.

Kuijpers TW, Vossen MT, Gent MR, Davin JC, Roos MT, Wertheim-van Dillen PM, Weel JF, Baars PA, van Lier RA. Frequencies of circulating cytolytic, CD45RA$^+$CD27-, CD8$^+$ T lymphocytes depend on infection with CMV. J Immunol 2003; 170(8):4342-8.

Kumpel BM, de Haas M, Koene HR, van de Winkel JGJ, Goodrick MJ. Clearance of red cells by monoclonal IgG3 anti-D *in vivo* is affected by the VF polymorphism of Fc gamma RIIIa (CD16). Clin Exp Immunol 2003; 132(1):81-6.

Kwa D, Boeser-Nunnink B, Schuitemaker H. Lack of evidence for an association between a polymorphism in CX3CR1 and the clinical course of HIV infection or virus phenotype evolution. AIDS 2003; 17(5):759-61.

Kwa D, van Rij RP, Boeser-Nunnink B, Vingerhoed J, Schuitemaker H. Association between an interleukin-4 promoter polymorphism and the acquisition of CXCR4 using HIV-1 variants. AIDS 2003; 17(7):981-5.

Kwa D, Vingerhoed J, Boeser-Nunnink B, Schuitemaker H. Increased *in vitro* cytopathicity of CC chemokine receptor 5-restricted human immunodeficiency virus type 1 primary isolates correlates with a progressive clinical course of infection. J Infect Dis 2003; 187(9):1397-403.

Kwa MSG, Van Schaik IV, De Jonge RR, Brand A, Kalaydjieva L, Van Belzen N, Vermeulen M, Baas F. Autoimmunoreactivity to Schwann cells in patients with inflammatory neuropathies. Brain 2003; 126:361-75.

Lefrere JJ, Allain JP, Prati D, Sauleda S, Reesink H. West Nile virus and blood donors. Lancet 2003; 361(9374):2083-4.

Lelkens CC, Noorman F, Koning JG, Truijens-de Lange R, Stekkinger 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.

Levering WH, Wind H, Hooijkaas H, Sintnicolaas K, Brando B, Gratama JW. Flow cytometric screening for HLA-B27 on peripheral blood lymphocytes. J Biol Regul Homeost Agents 2003; 17(3): 241-6.

Levering WH, Wind H, Sintnicolaas K, Hooijkaas H, Gratama JW. Flow cytometric HLA-B27 screening: Cross-reactivity patterns of commercially available anti-HLA-B27 monoclonal antibodies with other HLA-B antigens. Cytometry 2003; 54B(1):28-38.

Lie WJ, Homburg CH, Kuijpers TW, Knol EF, Mul FP, Roos D, Tool AT. Regulation and kinetics of platelet-activating factor and leukotriene C4 synthesis by activated human basophils. Clin Exp Allergy 2003; 33(8):1125-34.

Lopuhaa CE, de Riemer MJ, Out TA, Sjamsoedin DH, Aalberse RC, Jansen HM, van der Zee JS. Comparison of allergen-induced late inflammatory reactions in the nose and in the skin in house dust mite-allergic patients with or without asthma. Int Arch Allergy Immunol 2003; 130(4):266-74.

Mackus WJ, Frakking FN, Grummels A, Gamadia LE, De Bree GJ, Hamann D, van Lier RA, van Oers MH. Expansion of CMV-specific $CD8^+CD45RA^+$. Blood 2003; 102(3):1057-63.

Maianski NA, Roos D, Kuijpers TW. Tumor necrosis factor alpha induces a caspase-independent death pathway in human neutrophils. Blood 2003; 101(5):1987-95.

Masuda M, Morimoto T, de Haas M, Nishimura N, Nakamoto K, Okuda K, Komiyama Y, Ogawa R, Takahashi H. Increase of soluble FcgRIIIa derived from natural killer cells and macrophages in plasma from patients with rheumatoid arthritis. J Rheumatol 2003; 30(9):1911-7.

Meij P, van Esser JW, Niesters HG, van Baarle D, Miedema F, Blake N, Rickinson AB, Leiner I, Pamer E, Lowenberg B, Cornelissen JJ, Gratama JW. Impaired recovery of Epstein-Barr virus (EBV)-specific $CD8^+$ T lymphocytes after partially T-depleted allogeneic stem cell transplantation may identify patients at very high risk for progressive EBV reactivation and lymphoproliferative disease. Blood 2003; 101(11):4290-7.

Metcalfe P, Watkins NA, Ouwehand WH, Kaplan C, Newman P, Kekomaki R, de Haas M, Aster R, Shibata Y, Smith J, Kiefel V, Santoso S. Nomenclature of human platelet antigens. Vox Sang 2003; 85(3):240-5.

Mohammadi T, Reesink HW, Vandenbroucke-Grauls CM, Savelkoul PH. Optimization of real-time PCR assay for rapid and sensitive detection of eubacterial 16S ribosomal DNA in platelet concentrates. J Clin Microbiol 2003; 41(10):4796-8.

Mulherin SA, O'Brien TR, Ioannidis JPA, Goedert JJ, Buchbinder SP, Coutinho RA, Jamieson BD, Meyer L, Michael NL, Pantaleo G, Rizzardi GP, Schuitemaker H, Sheppard HW, Theodorou ID, Vlahov D, Rosenberg PS. Effects of CCR5-Delta 32 and CCR2-64I alleles on HIV-1 disease progression: the protection varies with duration of infection. AIDS 2003; 17(3):377-87.

Naldini A, Aarden L, Pucci A, Bernini C, Carraro F. Inhibition of interleukin-12 expression by alpha-thrombin in human peripheral blood mononuclear cells: a potential mechanism for modulating Th1/Th2 responses. Br J Pharmacol 2003; 140(5):980-6.

Nauta AJ, Bottazzi B, Mantovani A, Salvatori G, Kishore U, Schwaeble WJ, Gingras AR, Tzima S, Vivanco F, Egido J, Tijsma O, Hack CE, Daha MR, Roos A. Biochemical and functional characterization of the interaction between pentraxin 3 and C1q. Eur J Immunol 2003; 33(2):465-73.

Netelenbos T, van den Born J, Kessler FL, Zweegman S, Merle PA, van Oostveen JW, Zwaginga JJ, Huijgens PC, Drager AM. Proteoglycans on bone marrow endothelial cells bind and present SDF-1 towards hematopoietic progenitor cells. Leukemia 2003; 17(1):175-84.

Niessen HW, Hack CE. C-reactive protein and the risk of cardiovascular mortality. Am J Med 2003; 114(3):241-2.

Nijmeijer R, Lagrand WK, Lubbers YT, Visser CA, Meijer CJ, Niessen HW, Hack CE. C-reactive protein activates complement in infarcted human myocardium. Am J Pathol 2003; 163(1):269-75.

Nijmeijer R, Willemsen M, Meijer CJ, Visser CA, Verheijen RH, Gottlieb RA, Hack CE, Niessen HW. Type II secretory phospholipase A2 binds to ischemic flip-flopped cardiomyocytes and subsequently induces cell death. Am J Physiol Heart Circ Physiol 2003; 285(5):H2218-24.

Postma MJ, van de Watering LMG, de Vries R, Versmoren D, van Hulst M, Tobi H, van der Poel CL, Brand A. Cost-effectiveness of leucocyte depletion of red-cell transfusions for patients undergoing cardiac surgery. Vox Sang 2003; 84(1):65-7.

Price LS, Langeslag M, ten Klooster JP, Hordijk PL, Jalink K, Collard JG. Calcium signaling regulates translocation and activation of Rac. J Biol Chem 2003; 278(41):39413-21.

Rada BK, Geiszt M, van Bruggen R, Nemet K, Roos D, Ligeti E. Calcium signalling is altered in myeloid cells with a deficiency in NADPH oxidase activity. Clin Exp Immunol 2003; 132(1):53-60.

Radder CM, Brand A, Kanhai HHH. Will it ever be possible to balance the risk of intracranial haemorrhage in fetal or neonatal alloimmune thrombocytopenia against the risk of treatment strategies to prevent it? Vox Sang 2003; 84:318-25.

Reesink HW, Engelfriet CP, Muylle L, Wendel S, Dickmeiss E, Krusius T, Maki T, Lin CK, O'Riordan J, Prati D, Rebulla P, Shirato T, Nakajima K, Dupuis HM, Flanagan P, Carasa MA, Gallastegui RA, Turek P, Hewitt P, Bernat JL, Bianco C, Dodd RY, Klein HG. Future counselling of donors and recipients of blood products concerning prion-related diseases. Vox Sang 2003; 85(2):126-48.

Reumaux D, de Boer M, Meijer AB, Duthilleul P, Roos D. Expression of myeloperoxidase (MPO) by neutrophils is necessary for their activation by anti-neutrophil cytoplasm autoantibodies (ANCA) against MPO. J Leukoc Biol 2003; 73(6):841-9.

Reumaux D, Kuijpers TW, Hordijk PL, Duthilleul P, Roos D. Involvement of Fcgamma receptors and beta2 integrins in neutrophil activation by anti-proteinase-3 or anti-myeloperoxidase antibodies. Clin Exp Immunol 2003; 134(2):344-50.

Rijnders RJ, van der Luijt RB, Peters ED, Goeree JK, van der Schoot CE, Ploos Van Amstel JK, Christiaens GC. Earliest gestational age for fetal sexing in cell-free maternal plasma. Prenat Diagn 2003; 23(13):1042-4.

Rohlena J, Kolkman JA, Boertjes RC, Mertens K, Lenting PJ. Residues Phe342-Asn346 of activated coagulation factor IX contribute to the interaction with low density lipoprotein receptor-related protein. J Biol Chem 2003; 278(11):9394-401.

Romani de Wit T, de Leeuw HP, Rondaij MG, de Laaf RT, Sellink E, Brinkman HJ, Voorberg J, van Mourik JA. Von Willebrand factor targets IL-8 to Weibel-Palade bodies in an endothelial cell line. Exp Cell Res 2003; 286(1):67-74.

Romani de Wit T, Fijnheer R, Brinkman HJ, Kersting S, Hene RJ, van Mourik JA. Endothelial cell activation in thrombotic thrombocytopenic purpura (TTP): a prospective analysis. Br J Haematol 2003; 123(3):522-7.

Romani de Wit T, Rondaij MG, Hordijk PL, Voorberg J, van Mourik JA. Real-time imaging of the dynamics and secretory behavior of weibel-palade bodies. Arterioscler Thromb Vasc Biol 2003; 23(5): 755-61.

Roos A, Bouwman LH, Munoz J, Zuiverloon T, Faber-Krol MC, Fallaux-van den Houten FC, Klar-Mohamad N, Hack CE, Tilanus MG, Daha MR. Functional characterization of the lectin pathway of complement in human serum. Mol Immunol 2003; 39(11): 655-68.

Roos D, van Bruggen R, Meischl C. Oxidative killing of microbes by neutrophils. Microbes Infect 2003; 5(14):1307-15.

Sankatsing SU, van Praag RM, van Rij RP, Rientsma R, Jurriaans S, Lange JM, Prins JM, Schuitemaker H. Dynamics of the pool of infected resting CD4 HLA-DR-T lymphocytes in patients who started a triple class five-drug antiretroviral regimen during primary HIV-1 infection. Antivir Ther 2003; 8(2):137-42.

Schatz S, Turecek PL, Fiedler C, Zimmermann K, Gritsch H, Voorberg J, Schwarz HP, Dorner F, Scheiflinger F. Evaluation of the haemostatic potential of factor VIII-heparin cofactor II hybrid proteins in a mouse model. Br J Haematol 2003; 123(4):692-5.

Scherpbier HJ, Hilhorst MI, Kuijpers TW. Liver failure in a child receiving highly active antiretroviral therapy and voriconazole. Clin Infect Dis 2003; 37(6):828-30.

Schipper LF, Brand A, Reniers N, Melief CJ, Willemze R, Fibbe WE. Differential maturation of megakaryocyte progenitor cells from cord blood and mobilized peripheral blood. Exp Hematol 2003; 31(4):324-30.

Sentjens R, Basaras M, Simmonds P, Vrielink H, Reesink H. HGV/GB virus C transmission by blood components in patients undergoing open-heart surgery. Transfusion 2003; 43(11):1558-62.

Sturk-Maquelin KN, Nieuwland R, Romijn FP, Eijsman L, Hack CE, Sturk A. Pro- and non-coagulant forms of non-cell-bound tissue factor *in vivo*. J Thromb Haemost 2003; 1(9):1920-6.

Suharti C, van Gorp EC, Dolmans WM, Setiati TE, Hack CE, Djokomoeljanto R, van der Meer JW. Cytokine patterns during dengue shock syndrome. Eur Cytokine Netw 2003; 14(3):172-7.

Tacx AN, Groeneveld AB, Hart MH, Aarden LA, Hack CE. Mannan binding lectin in febrile adults: no correlation with microbial infection and complement activation. J Clin Pathol 2003; 56(12): 956-9.

Tans G, van Hylckama Vlieg A, Thomassen MC, Curvers J, Bertina RM, Rosing J, Rosendaal FR. Activated protein C resistance determined with a thrombin generation-based test predicts for venous thrombosis in men and women. Br J Haematol 2003; 122(3):465-70.

Ten Cate H, Brandjes DP, Smits PH, van Mourik JA. The role of platelets in venous thrombosis: a patient with Glanzmann's thrombasthenia and a factor V Leiden mutation suffering from deep venous thrombosis. J Thromb Haemost 2003; 1(2):394-5.

Ten Tusscher GW, Steerenberg PA, van Loveren H, Vos JG, von dem Borne AE, Westra M, van der Slikke JW, Olie K, Pluim HJ, Koppe JG. Persistent hematologic and immunologic disturbances in 8-year-old Dutch children associated with perinatal dioxin exposure. Environ Health Perspect 2003; 111(12):1519-23.

Terreehorst I, Hak E, Oosting AJ, Tempels-Pavlica Z, de Monchy JG, Bruijnzeel-Koomen CA, Aalberse RC, Gerth van Wijk R. Evaluation of impermeable covers for bedding in patients with allergic rhinitis. N Engl J Med 2003; 349(3):237-46.

Tesselaar K, Arens R, van Schijndel GM, Baars PA, van der Valk MA, Borst J, van Oers MH, van Lier RA. Lethal T-cell immunodeficiency induced by chronic costimulation via CD27-CD70 interactions. Nat Immunol 2003; 4(1):49-54.

Tesselaar K, Xiao Y, Arens R, van Schijndel GM, Schuurhuis DH, Mebius RE, Borst J, van Lier RA. Expression of the murine CD27 ligand CD70 *in vitro* and *in vivo*. J Immunol 2003; 170(1):33-40.

Thomas I, Di Giambattista M, Gerard C, Mathys E, Hougardy V, Latour B, Branckaert T, Laub R. Prevalence of human erythrovirus B19 DNA in healthy Belgian blood donors and correlation with specific antibodies against structural and non-structural viral proteins. Vox Sang 2003; 84(4):300-7.

Tsegaye A, Wolday D, Otto S, Petros B, Assefa T, Alebachew T, Hailu E, Adugna F, Measho W, Dorigo W, Fontanet AL, van Baarle D, Miedema F. Immunophenotyping of blood lymphocytes at birth, during childhood, and during adulthood in HIV-1-uninfected Ethiopians. Clin Immunol 2003; 109(3):338-46.

Ujhelly O, Özvegy C, Várady G, Cervenak J, Homolya L, Grez M, Scheffer G, Roos D, Bates SE, Várady A, Sarkadi B, Német K. Application of a human multidrug transporter (ABCG2) variant as selectable marker in gene transfer to progenitor cells. Hum Gene Ther 2003; 14(4):403-12.

Ulfman LH, Joosten DP, van Aalst CW, Lammers JW, van de Graaf EA, Koenderman L, Zwaginga JJ. Platelets promote eosinophil adhesion of patients with asthma to endothelium under flow conditions. Am J Respir Cell Mol Biol 2003; 28(4):512-9.

Van Buul JD, Voermans C, van Gelderen J, Anthony EC, van der Schoot CE, Hordijk PL. Leukocyte-endothelium interaction promotes SDF-1-dependent polarization of CXCR4. J Biol Chem 2003; 278(32):30302-10.

Van de Watering L, Hermans J, Witvliet M, Versteegh M, Brand A. HLA and RBC immunization after filtered and buffy coat-depleted blood transfusion in cardiac surgery: a randomized controlled trial. Transfusion 2003; 43(6):765-71.

Van de Wetering MD, Kuijpers TW, Taminiau JA, ten Kate FJ, Caron HN. Pseudomembranous and neutropenic enterocolitis in pediatric oncology patients. Support Care Cancer 2003; 11(9): 581-6.

Van der Poel CL, Schipperus MR. The emerging Dutch Hemovigilance system: voluntary and state-of-the-art. Transfus Altern Transfus Med 2003; 5(1):256-9.

Van der Schoot CE, Tax GH, Rijnders RJ, de Haas M, Christiaens GC. Prenatal typing of Rh and Kell blood group system antigens: the edge of a watershed. Transfus Med Rev 2003; 17(1):31-44.

Van Hennik PB, ten Klooster JP, Halstead JR, Voermans C, Anthony EC, Divecha N, Hordijk PL. The C-terminal domain of Rac1 contains two motifs that control targeting and signaling specificity. J Biol Chem 2003; 278(40):39166-375.

Van Manen HJ, Uzunbajakava N, van Bruggen R, Roos D, Otto C. Resonance Raman imaging of the NADPH oxidase subunit cytochrome b558 in single neutrophilic granulocytes. J Am Chem Soc 2003; 125(40):12112-3.

Van Nieuwenhuijze AE, van Lopik T, Smeenk RJ, Aarden LA. Time between onset of apoptosis and release of nucleosomes from apoptotic cells: putative implications for systemic lupus erythematosus. Ann Rheum Dis 2003; 62(1):10-4.

Van Rhenen D, Gullikssson H, Cazenave JP, Pamphilon D, Ljungman P, Kluter H, Vermeij H, Kappers-Klunne M, de Greef G, Laforet M, Lioure B, Davis K, Marblie S, Mayaudon V, Flament J, Conlan M, Lin L, Metzel P, Buchholz B, Corash L. Transfusion

of pooled buffy coat platelet components prepared with photochemical pathogen inactivation treatment: the euroSPRITE trial. Blood 2003; 101(6):2426-33.

Van Rij RP, Hazenberg MD, van Benthem BH, Otto SA, Prins M, Miedema F, Schuitemaker H. Early Viral Load and CD4(+) T Cell Count, But Not Percentage of CCR5(+) or CXCR4(+) CD4(+) T Cells, Are Associated with R5-to-X4 HIV Type 1 Virus Evolution. AIDS Res Hum Retroviruses 2003; 19(5):389-98.

Van Rij RP, Worobey M, Visser JA, Schuitemaker H. Evolution of R5 and X4 human immunodeficiency virus type 1 gag sequences *in vivo*: evidence for recombination. Virology 2003; 314(1):451-9.

Van Strien RT, Koopman LP, Kerkhof M, Oldenwening M, de Jongste JC, Gerritsen J, Neijens HJ, Aalberse RC, Smit HA, Brunekreef B. Mattress encasings and mite allergen levels in the Prevention and Incidence of Asthma and Mite Allergy study. Clin Exp Allergy 2003; 33(4):490-5.

Van Wetering S, van den Berk N, van Buul JD, Mul FP, Lommerse I, Mous R, ten Klooster JP, Zwaginga JJ, Hordijk PL. VCAM-1-mediated Rac signaling controls endothelial cell-cell contacts

and leukocyte transmigration. Am J Physiol Cell Physiol 2003; 285(2):C343-52.

Van Woensel JB, Lutter R, Biezeveld MH, Dekker T, Nijhuis M, van Aalderen WM, Kuijpers TW. Effect of dexamethasone on tracheal viral load and interleukin-8 tracheal concentration in children with respiratory syncytial virus infection. Pediatr Infect Dis J 2003; 22(8):721-6.

Vossebeld PJ, Tissing MH, van den Berg HM, Leebeek FW, Goede-Bolder A, Novakova IRO, Gerrits WB, Peters M, Koopman MM, Faber A, Hiemstra H, Grob P, Strengers PF. *In vivo* recovery and safety of human factor VIII product AAFACT in patients with haemophilia A. Haemophilia 2003; 9(2):157-63.

Vossen MT, Gent MR, Davin JC, Baars PA, Wertheim-van Dillen PM, Weel JF, Roos MT, van Baarle D, Groothoff J, van Lier RA, Kuijpers TW. Spontaneous outgrowth of EBV-transformed B-cells reflects EBV-specific immunity *in vivo*; a useful tool in the follow-up of EBV-driven immunoproliferative disorders in allograft recipients. Transpl Int 2004; 17:89-96.

Wahyuni S, Van Ree R, Mangali A, Supali T, Yazdanbakhsh M, Sartono E. Comparison of an enzyme linked immunosorbent assay (ELISA) and a radioallergosorbent test (RAST) for detection of IgE antibodies to Brugia malayi. Parasite Immunol 2003; 25(11-12):609-14.

Widdowson MA, Jaspers WJM, van der Poel WHM, Verschoor F, Husman AMD, Winter HLJ, Zaaijer HL, Koopmans M. Cluster of cases of acute hepatitis associated with hepatitis E virus infection acquired in the Netherlands. Clinical Infectious Diseases 2003; 36(1):29-33.

Willekens FLA, Roerdinkholder-Stoelwinder B, Groenen-Dopp YAM, Bos HJ, Bosman GJCGM, van den Bos AG, Verkleij AJ, Werre JM. Hemoglobin loss from erythrocytes *in vivo* results from spleen-facilitated vesiculation. Blood 2003; 101(2):747-51.

Zeerleder S, Caliezi C, van Mierlo G, Eerenberg-Belmer A, Sulzer I, Hack CE, Wuillemin WA. Administration of C1 inhibitor reduces neutrophil activation in patients with sepsis. Clin Diagn Lab Immunol 2003; 10(4):529-35.

Zeerleder S, Zürcher Zenklusen R, Hack CE, Wuillemin WA. Disseminated intravascular coagulation in meningococcal sepsis. Case 7. Hamostaseologie 2003; 23(3):125-30.

Zeerleder S, Zwart B, Wuillemin WA, Aarden LA, Groeneveld AB, Caliezi C, van Nieuwenhuijze AE, van Mierlo GJ, Eerenberg AJ, Lammle B, Hack CE. Elevated nucleosome levels in systemic inflammation and sepsis. Crit Care Med 2003; 31(7):1947-51.

Zwaginga JJ, Doevendans P. Stem cell-derived angiogenic/vasculogenic cells: possible therapies for tissue repair and tissue engineering. Clin Exp Pharmacol Physiol 2003; 30(11):900-8.

Miscellaneous publications

Alphabetically, first author

Aarden L.A. Immunologische overeenkomsten en verschillen tussen TNF-blokkers Reumatologie actueel jaargang 3 nr. 5 november 2003.

Aarden L.A. Immunologische overeenkomsten en verschillen tussen TNF-blokkers Gastro actueel jaargang 7 nr. 13, december 2003.

Akkerdaas JH, Wensing M, Knulst AC, Aalberse RC, Hefle SL, van Ree R. *In vitro* and *in vivo* characterization of hazelnut skin prick test extracts. Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A M. 2003; (94):87-95; discussion 96.

Bos HJ, Van der Poel CL. Veilig bloed voor risicopatiënten dankzij Parvovirus test. Infectieziekten bulletin 2003; 14(7):236-7.

Beckers EA, Dinkelaar RB, te Boekhorst PA, van Ingen HE, van Rhenen DJ. Meldingen over transfusie-incidenten: ervaringen in het eerste jaar van hemovigilantie in de regio van de voormalige Bloedbank ZWN Rotterdam [Reports of transfusion incidents: experiences from the first year of hemovigilance in the region of the former ZWN (South West Netherlands) blood bank in Rotterdam]. Ned Tijdschr Geneeskd 2003; 147(31):1508-12.

Brand A, Claas FHJ, Tomson TAS, Witvliet M. Detection of platelet-reactive antibodies in patients who are refractory to platelet transfusions, and the selection of compatible donors. Vox Sanguinis International Forum 2003; 84:73-88.

De Haas M, Schoot CE, Overbeeke M, Koelewijn J, Bonsel G, Vrijkotte T. International forum: 7. Vox Sang 2003; 85(4):333-4.

De Korte D. Implementation of a Screening System for Bacteria Detection. In: Brecher ME, ed. Bacterial and Parasitic Contamination of Blood Components. Bethesda, MD: AABB Press, 2003.

Dijkstra-Tiekstra MJ, Schrijver JG, Meer PF van der, Laporte RF, Gratama JW, Levering WHBM, Delden CJ van, Wildt-Eggen J de. Crossover study of three methods for low-level white blood cell counting on two types of flow cytometer Cytometry Part B (Clin Cytometry) 2003; 54B:39-45.

Engelfriet CP, Reesink HW. Detection of platelet-reactive antibodies in patients who are refractory to platelet transfusions, and the selection of compatible donors. International Forum. Vox Sang 2003; 84:73-88.

Engelfriet CP, Reesink HW, Judd WJ, Ulander VM, Kuosmanen M, Koskinen S, Rouger P, Morelati F, Tantalo V, Fujii T, de Haas M, van der Schoot CE, Overbeeke M, Koelewijn J, Bonsel G, Vrijkotte T, Zupanska B, Martin-Vega C, Parra LR, de Silva M, Contreras M, Panzer S, Ulm B, Mayr WR. International forum. Current status of immunoprophylaxis with anti-D immunoglobin. Vox Sang 2003; 85(4):328-37.

Hordijk PL, van Wetering S. VCAM-1-mediated Rac signaling controls leukocyte transmigration. Faseb Journal 2003; 17(5): A1189.

Jansen AJG, Essink-Both ML, Hop WCJ, Beckers EAM, Van Rhenen DJ. Transfusiebeleid en kwaliteit van leven. Ned Tijdschrift Klin Chemie 2003; 28:248-52.

Lelie PN, van Drimmelen AAJ, Weusten J. Risk of transfusion-transmitted infections by NAT-negative blood – Reply. Transfusion 2003; 43(6):831.

Lindeboom JA, Prins JM, Peeters MF, Kuijper EJ, Kuijpers TW. Mycobacterium haemophilium als veroorzaker van lymphadenitis colli bij een niet-zieke jongen (Mycobacterium haemophilum as the cause of lymphadenitis in the neck in an otherwise healthy boy). Ned Tijdschr Geneeskd 2003; 147(46):2305-6.

Pietersz RNI, Engelfriet CP, Reesink HW. Detection of bacterial contamination of platelet concentrates. International Forum. Vox Sang 2003; 85:224-39.

Pietersz RN, Engelfriet CP, Reesink H. Detection of bacterial contamination of platelet concentrates: International forum. Vox Sang 2003; 85(3):234.

Reesink HW, Engelfriet CP Future counselling of donors and recipients of blood products concerning prion-related diseases. International Forum. Vox Sang 2003; 85:126-48.

Ruys ThA, Reesink HW, Lange JMA. Chronische hepatitis C virusinfecties bij mensen met een HIV-infectie. Ned Tijdschr Geneeskd 2003; 147:2056-60.

Silva M de, Engelfriet CP, Reesink HW. Current status of immunoprophylaxis with anti-D immunoglobulin. International Forum. Vox Sang 2003; 85:328-37.

Smeenk R, Wiik A. EULAR Standing Committee of Investigative Rheumatology: report of activities 2002-3. Ann Rheum Dis 2003; 62(12):1251.

Strengers PFW, Faber J, Rossi U. Haemovigilance : Proceedings of the SIMTI/ESTM course, Piacenza (Italy), 20th-21st June 2003. Strengers PFW, Faber J, Salpeteur C. Haemovigilance in clinical care: uniform reporting form for serious adverse events. Haemovigilance: Proceedings of the SIMTI/ESTM course, Piacenza (Italy), 20th-21st June 2003. 2003;143-7.

Strengers PFW, Kallenberg CGM, Cohen Tervaert JW, Kuijpers TW, Kuis W, Schellekens PTA. De rol van intraveneuze immunoglobulinen bij auto-immunziekten (Het gebruik van intraveneuze immunoglobulinen (IVIG) in de klinsche praktijk, Sanquin Symposia 2001–2002). Amsterdam. Sanquin. 2003; 2e herz.:9-48.

Strengers PFW, Meulenbroek AJ, Bleeker W, Visser LA, van Doorn PA, van den Berg LH, Meilof JF. Huidige inzichten in de klinische aspecten van intraveneus immunoglobuline bij neurologische aandoeningen (Het gebruik van intraveneuze immunoglobulinen (IVIG) in de klinsche praktijk, Sanquin Symposia 2001–2002). Amsterdam. Sanquin. 2003; 2e herz.:9-64.

Strengers PFW, van der Meer JWM, Kuijpers TW, Bredius RGM, de Baets F, Sanders EAM, Kallenberg CGM. Kliniek en behandeling van primaire en secundaire immuundeficiënties; deel 2; [Het gebruik van intraveneuze immunoglobulinen (IVIG) in de klinische praktijk; Sanquin Symposia 2001 – 2002]. Amsterdam: Sanquin, 2003.

Strengers PFW. Haemovigilance in the light of the new European Union's Blood directive. Haemovigilance: Proceedings of the SIMTI/ESTM course, Piacenza (Italy), 20th-21st June 2003 2003;7-9.

Strengers PFW. Haemovigilance, an important tool to improve transfusion practice. Transfusion Alternatives in Transfusion Medicine.2003; 5-2 suppl.:26-7.

Strengers PFW. Models for education in different levels of transfusion medicine. Blood Bank Transf Med 2003; 1(1):S241-3.

Strengers PFW. The EU Blood Directive, consequences for national blood transfusion organisations. Lijec Vjesn 2003; 125 suppl.3: 16-7.

Strengers PFW. The European Haemovigilance Network (EHN): conclusions of the last Seminar in Amsterdam (6-7 February 2003). Haemovigilance: Proceedings of the SIMTI/ESTM course, Piacenza (Italy), 20th-21st June 2003 2003; 57-9.

Van de Watering LMG, Van Hilten JA, Brand A. Bloedgebruik per patientencategorie. Ned Tijdschrift Klin Chem 2003; 28:295-8.

Van der Meer P, van Gilst WH, van Veldhuisen DJ. Anemia and congestive heart failure. Circulation 2003; 108(6):e41-2.

Van der Poel CL, Schipperus MR. Veilige bloedtransfusie, Nederlands hemovigilantiesysteem van start. Medisch contact 7 maart 2003; 58 (10):392-3.

Van der Poel CL, Seifried E, Schaasberg W. Author's reply. Vox Sang 2003; 85(1):50-1.

Van Ree R, Yazdanbakhsh M. Parallels between parasite infections and specific immunotherapy. Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A M. 2003;(94):260-3; discussion 263-6.

Van Ree R. The CREATE project: a new beginning of allergen standardization based on mass units of major allergens. Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A M. 2003;(94): 70-3; discussion 74-5.

Van Rhenen DJ. Pathogeen reductie van bloedproducten en de kliniek. Ned Tijdschrift Klin Chemie 2003; 28:223-6.

Verhoeven AJ. Hemoglobine-oplossingen: komt het er nog van? Ned Tijdschr Klin Chem 2003; 28:226-30.

Vossebeld PJM, Eijkhout HW, van Aken WG. Blood, blood components, plasma, and plasma products. Side Effects of Drugs, Annual 26 2003; (33):363-73.

PhD theses

Thalia Romani de Wit

20 February 2003

Weibel-Palade bodies. An exciting way out!

University of Utrecht

Promotor: Prof K Mertens (Faculty of Farmaceutical Sciences, University of Utrecht and Sanquin Research at CLB, Amsterdam)

Co-promotor: Dr JA van Mourik (Sanquin Research at CLB, Amsterdam)

Christof Meischl

1 April 2003

NADPH oxidases and mutation analysis

University of Amsterdam

Promotor: Prof D Roos (Sanquin Research at CLB, Amsterdam)

Wendy Bril

22 May 2003

Factor VIII inhibitors in mild haemophilia A

University of Utrecht

Promotor: Prof K Mertens (Faculty of Pharmaceutical Sciences, University of Utrecht and Sanquin Research at CLB, Amsterdam) and Prof RAW van Lier (AMC, Amsterdam)

Copromotor: Dr JJ Voorberg (Sanquin Research at CLB, Amsterdam)

Niels Bovenschen

16 June 2003

The Interaction between Coagulation Factor VIII and Its Clearance Receptors

University of Utrecht

Promotor: Prof K Mertens (Faculty of Pharmaceutical Sciences, University of Utrecht and Sanquin Research at CLB, Amsterdam)

Copromotors: Dr AB Meijer and Dr PJ Lenting

Erica van Oort

19 September 2003

Pros and Cons of Natural and Recombinant Allergens

University of Amsterdam

Promotor: Prof RC Aalberse (Sanquin Research at CLB, Amsterdam)

Copromotor: Dr R van Ree (Sanquin Research at CLB, Amsterdam)

Ineke Bos

21 November 2003

C1-Inhibitor potentiation by glycosaminiglycans

University of Amsterdam

Promotor: Prof CE Hack (Sanquin Research at CLB, Amsterdam) en prof JP Abrahams

Copromotor: Dr E Eldering

Academic Staff index

Index on key words

Colophon

Further information may be obtained from
Jan Willem Smeenk
Corporate Staff
Sanquin Blood Supply Foundation
P.O. Box 9892
NL-1006 AN Amsterdam
The Netherlands
T +31 20 512 30 00
F +31 20 512 33 03
jw.smeenk@sanquin.nl
www.research.sanquin.nl

Editors
Prof F Miedema PhD
Prof DJ van Rhenen MD PhD
JW Smeenk MSc
ANCJ de Regt MA

Design
Total Identity, The Hague

Printing
Ando bv, The Hague