Red Blood Cell Aspects, Defects and Prospects

14 & 15 April 2005
De Rode Hoed
Amsterdam
The Netherlands
Sanquin Spring Seminars
Red Blood Cell Aspects, Defects and Prospects

Chairman: Prof. Dirk Roos PhD, Amsterdam, The Netherlands

14 & 15 April 2005
De Rode Hoed, Amsterdam, The Netherlands

Special support is given by:
Baxter Health Care, Utrecht, The Netherlands,
Fresenius Hemocare, Emmer Compascuum, The Netherlands,
Sanquin Blood Supply Foundation, Amsterdam, The Netherlands

www.sss.sanquin.nl
Scientific Committee
Prof. Dirk Roos PhD, Chairman
Arthur Verhoeven PhD
Prof. Lucien Aarden PhD
Prof. Anneke Brand MD, PhD
Cees van der Poel MD, PhD
Prof. Dick van Rhenen MD, PhD
Ellen van der Schoot MD, PhD

Organizing Committee
Jeroen de Wit, Chairman
Ingeborg van der Heijden
Cees van der Poel
Wies Schueler
Jan Willem Smeenk

Seminar Secretariat
Eurocongres Conference Management
J. van Goyenkade 11
1075 HP Amsterdam, The Netherlands
Tel: +31-20-679.3411, Fax: +31-20-673.7306
sanquin@eurocongres.com
http://www.sss.sanquin.nl

Venue
De Rode Hoed
Keizersgracht 102
1015 CV Amsterdam, The Netherlands
Tel: +31-20-638.5606, Fax: +31-20-625.4452
Scientific programme,
Thursday, April 14th, 2005

09.00 hrs.: Start of registration

09.00 – 10.30 hrs: Coffee

10.30 hrs: Opening of seminar by Ernest Briët, member Executive Board Sanquin

**Session I: RBC structure**
Chair: C. Ellen van der Schoot (Amsterdam, The Netherlands)
10.35 – 11.10 hrs: Structure and function of the red cell membrane and its cytoskeleton
Jean Delaunay (Paris, France)
11.10 – 11.45 hrs: Structure of the Rh complex
Neil Avent (Bristol, UK)
11.45 – 12.00 hrs: Ankyrin gene in Hereditary Spherocytosis
Eric Vermeer (The Hague, The Netherlands)
12.00 – 13.15 hrs: Lunch break

**Session II: RBC metabolism**
Chair: Ernest Beutler (La Jolla, USA)
13.15 – 13.50 hrs: Enzyme deficiencies in RBC
Wouter van Solinge (Utrecht, The Netherlands)
13.50 – 14.25 hrs: Interaction between NO and hemoglobin
Mark Gladwin (Bethesda, MD, USA)
14.25 – 14.40 hrs: ADP receptors of the P2Y13 type on RBC
Martin Olsson (Lund, Sweden)
14.40 – 14.55 hrs: Regulation of PS exposure in RBC
Arthur Verhoeven (Amsterdam, The Netherlands)
14.55 – 15.20 hrs: Tea break

**Session III: Aging and removal of RBC**
Chair: Dirk Roos (Amsterdam, The Netherlands)
15.20 – 15.45 hrs: Biochemical differences between young and old RBC
Giel Bosman (Nijmegen, The Netherlands)
15.45 – 16.00 hrs: IgM-IgG immune complexes in WAIHA
Dorothea Stahl (Münster, Germany)
16.00 – 16.15 hrs: PS exposure on RBC following GPC ligation
David Head (Bristol, UK)
Scientific programme,
Friday, April 15th, 2005

Session IV: RBC preservation and sterilization
Chair: Hans Loos (Amsterdam, The Netherlands)
16.15 – 16.50 hrs: Function of CD47 on RBC
    Per-Arne Oldenborg (Umeå, Sweden)
16.50 – 17.05 hrs: CD47 – an inducer of RBC apoptosis?
    Zoe Lee (Bristol, UK)

Session V: Iron metabolism and hereditary hemochromatosis
Chair: Sweder van Asbeck (Utrecht, The Netherlands)
08.30 – 09.05 hrs: Iron metabolism and RBC turnover
    Jo Marx (Utrecht, The Netherlands)
09.05 – 09.40 hrs: The penetrance of hereditary hemochromatosis:
    controlled biochemical and clinical observations in a normal population
    Ernest Beutler (La Jolla, CA, USA)
09.40 – 10.15 hrs: Coffee break

Session VI: Hemoglobin solutions
Chair: Arthur Verhoeven (Amsterdam, The Netherlands)
10.15 – 10.50 hrs: Hemoglobin-based RBC substitutes
    Bob Winslow (San Diego, CA, USA)
10.50 – 11.25 hrs: Local oxygenation by RBC and hemoglobin-based substitutes
    Can Ince (Amsterdam, The Netherlands)
11.25 – 11.40 hrs: Blood substitutes for clinical use
   Evguen Selivanov (St. Petersburg, Russia)

11.40 – 13.00 hrs: Lunch break

Session VII: Universal RBC
Chair: Masja de Haas (Amsterdam, The Netherlands)
13.00 – 13.35 hrs: PEGylated RBC as universal red cells
   Timothy Fisher (Los Angeles, CA, USA)
13.35 – 14.10 hrs: Enzymatically treated non-O RBC,
   Martin Olsson (Lund, Sweden)

14.10 – 14.45 hrs: Tea break

Session VIII: Therapy for RBC dysfunctions
Chair: Jaap Jan Zwaginga (Amsterdam, The Netherlands)
14.45 – 15.20 hrs: Modulation of inflammation in Sickle Cell Disease
   John D. Belcher (Minneapolis, USA)
15.20 – 15.55 hrs: Bone marrow transplantation in Sickle Cell Disease
   Christiane Vermylen (Brussels, Belgium)

Session IX: Blood saving practices
Chair: Leon Eijsman (Amsterdam, The Netherlands)
15.55 – 16.30 hrs: Blood saving in surgery
   Ron Speekenbrink (Amsterdam, The Netherlands)
16.30 – 17.05 hrs: Erythropoietin therapy
   Yves Bequin (Liege, Belgium)
17.05 – 17.20 hrs: Post-operative re-infusion of shed blood,
   Cynthia So-Osman (Leiden, The Netherlands)

17.20 hrs: Farewell reception
Structure and function of the red cell membrane and its skeleton

The plasma membrane is the only membrane of the red cell. It possesses the general features of plasma membranes. The lipid bilayer is studded with a variety of transmembrane proteins. On the opposite, the membrane skeleton is a distinctive feature of the erythrocyte. It represents a thick bidimensional network that runs beneath the inner leaflet of the bilayer. It provides the red cell with the resistance and the elastic deformability required in the arteries and the capillaries, respectively. Studies on lipid rafts within the lipid bilayer have opened a new field. They are sphingolipid- and cholesterol-rich membrane microdomains, insoluble in non-ionic detergents. Stomatin, flotillin-1 and -2, aquaporin-1, and some GPI-anchored proteins are concentrated in the rafts. The most important transmembrane proteins are band 3, or the anion exchanger, the glycophorins and the Rh polypeptides, RhD/CE, and related proteins. Between themselves and/or through their interaction with skeletal proteins, transmembrane proteins tend to assemble into complexes. (i) Stomatin and the flotillins organize in high-order oligomers. Aquaporin-1 appears as a tetramer. (ii) Band 3 forms dimers and tetramers. Through ankyrin, it tethers spectrin tetramers close to the central region of the latter, containing the dimer-dimer self-association site (spectrin β-chain C-terminal region, specifically). Spectrin is the major protein of the skeleton. Altogether, the whole assembly accounts for the ‘band 3 complex’ which also includes the more marginally situated protein 4.2. RhD/CE, the Rh-associated glycoprotein (RhAG), glycophorin B, CD47 and the LW protein (ICAM 4) confederate into the ‘Rh complex’. Both the band 3 complex and the Rh complex associate into a macrocomplex, based on contacts between protein 4.2 and CD47, and between RhD/CE, RhAG and ankyrin (as is currently known). It is assumed that such a macrocomplex fulfills some highly integrated functions, however they remain to be identified. (iii) Next to their extremities, spectrin tetramers are connected again to the lipid bilayer through another transmembrane protein, glycophorin C, but still not directly. There is a protein complex playing a dual role. Through one region of its molecule, protein 4.1 binds to spectrin (spectrin β-chain N-terminal region, specifically) in a ‘horizontal’ interaction, that also involves the following proteins : actin (oligomers), adducin, protein p55, tropomyosin, tropomodulin. Through another region of its molecule,
protein 4.1 is connected with glycophorin C and p55, achieving a ‘vertical’ interaction. Most of the above proteins have a wide tissue distribution. Depending on the cell type, a variety of isoforms are generated by means of alternative initiation of transcription, alternative splicing or alternative initiation of translation, among other mechanisms. Red cell diseases caused by mutations in the above protein are seldom associated with non-erythroid manifestations. Many proteins sticking out of the bilayer constitute the structural basis of red blood cell antigens, as will be detailed. A variety of molecular changes accounting for rare blood group phenotypes have been elucidated.
Structure Modelling of the Rh Complex

The three-dimensional configuration of the human Rh proteins and their antigens has promoted much debate since the cDNAs encoding the proteins were cloned in the early 1990s. The Rh complex comprises two different but closely related Rh protein family members, the Rh associated glycoprotein (RhAG) and one of two different Rh polypeptides (RhCE or RhD). RhAG is critical for this assembly of this complex. Defects in its structural gene RHAG causes the Rh null phenotype which is characterised by the complete absence of the Rh complex, and another red cell membrane protein, ICAM-4 and a marked reduction in the levels of glycophorin B and CD47.

The current model of RhAG/Rh assembly is that it is arranged as a tetramer, comprising an $\alpha_2\beta_2$ RhAG/RhCE or RhAG/RhD complex.

In 2000 RhAG was shown by yeast complementation studies to transport ammonium and its analogues. Thus, Rh proteins are members of the ammonium transport family, which has representatives in all forms of life. Recent work, published in September 2004 (Khademi et al.,) has presented a high-resolution crystal structure of the *E.coli* ammonium transporter AmtB and has presented a model for the transport of ammonia/ammonium across the *E.coli* membrane. The AmtB, like most known ammonium transport complexes is arranged as a trimer. The publication of the co-ordinates of the AmtB complex has enabled, by homology modelling, the prediction of the structure of the Rh complex which challenges the current tetramer model. The Rh proteins have been modelled, and predict a similar arrangement to the AmtB monomer with both intra- and extracellular vestibules, and a large endoloop that may bind cytoskeletal components. The extracellular vestibule is of sufficient dimensions to allow the penetration of anti-D paratopes, with some predicted epitopes being buried deep within the vestibule. The model generates interesting observations concerning the VS and compound Rh antigens Rh ce, Ce, cE and CE.
Ankyrin gene in Hereditary Spherocytosis

Introduction:
Hereditary spherocytosis (HS) is a congenital haemolytic anaemia, the severity of which varies from asymptomatic to severe condition, giving rise to symptoms including icterus, anaemia and splenomegaly. One of the erythrocyte membrane proteins is ankyrin-1 (ANK-1), belonging to a family of proteins coordinating interactions between various integral membrane proteins and cytoskeletal elements. In fact, the most common cause (~35 to 65%) of typical, dominant HS is caused by gene mutations in the erythrocyte isoform (band 2.1, Mr=210 kDa). The ANK-1 gene is composed of 42 exons, and the composite cDNA contains 5636 base pairs (1879 amino acids).

Aim of the study:
We started a program to analyse genetic mutations in 30 patients with suspected HS.

Methods:
We set up two approaches to identify mutants in order to develop a sensitive screening strategy for suspected HS patients: 1) sequencing of all 42 coding exons plus the 5’ untranslated/promoter region; 2) comparison of genomic DNA and cDNA for common ANK1 polymorphisms.

Results and Conclusions:
We screened 40 coding exons. Beside common polymorphisms we found two new missense mutations: exon 12 (R426W) and exon 34 (Y1386H). Furthermore, we found one stop mutation in exon 37 (R1488X) and two previously described mutations in the ankyrin-1 promotor. As a screening strategy, we started a comparison of polymorphisms in exon 26 and 39 of genomic DNA and cDNA to identify reduced expression of mRNA. Polymorphisms in exon 26 and 39 are frequent in our patient population (80%).
Enzyme Deficiencies in Red Blood Cells

Erythrocytes perform a variety of functions, the most important being the binding, transport, and delivery of oxygen to all tissues. To do so, they must be capable of passage through microcapillaries, which is achieved by modifications of the erythrocyte’s biconcave shape. This is possible because, unlike most other cells in the body, human erythrocytes lose the nucleus and organelles before entering the circulation from the marrow. In addition, remaining RNA in the reticulocyte is lost within the first 2 days in circulation, thereby making further protein synthesis no longer possible.

Normal human red cells survive in the circulation for approximately 120 days, using energy to maintain the electrolyte gradient between plasma and red cell cytoplasm, to keep hemoglobin, the sulfhydryl groups of the red cell enzymes and membrane proteins in the reduced state. Because of the absence of a nucleus and mitochondria, the red cell is incapable of generating energy via the (oxidative) Krebs cycle and depends mainly on the anaerobic conversion of glucose by the Embden-Meyerhof pathway (EMP or direct glycolytic pathway) and the oxidative hexose monophosphate pathway (HMP or pentose phosphate shunt).

Numerous red cell enzymes are involved in these pathways, thereby providing the cell with the necessary moles of ATP. Deficiencies of any of these red cell enzymes may result in impaired ATP generation and consequently loss of function of the erythrocyte.

By far the majority of these disorders are hereditary in nature, although acquired deficiencies have been described. Hereditary enzymatic defects in these pathways disturb the erythrocyte’s integrity, shorten its cellular survival, and produce chronic nonspherocytic hemolytic anemia (CNSHA). Deficiencies of some enzymes, however, may not lead to chronic hemolytic anemia, but to acute episodes of severe hemolysis when there is increased oxidative stress on the red cell (as in some types of glucose-6-phosphate dehydrogenase deficiency).

A number of these enzymes are expressed in other tissues as well but cause a notable deficiency predominantly in red blood cells because of the life span of the erythrocyte after the loss of protein synthesis. Once an enzyme is degraded or otherwise becomes nonfunctional, it cannot be replaced by new or other “compensating” proteins because of the lack of nucleus, mitochondria, ribosomes, and other cell organelles in mature red cells. Disorders have been described in...
the EMP, HMP, Rapoport-Luebering cycle, the glutathione pathway, the purine-pyrimidine metabolism and methemoglobin reduction. The genotype to phenotype relations in these enzymopathies have been poorly understood. The degree of hemolysis varies considerably, ranging from very mild and compensated anemia to neonatal death. During the last few years, a vast body of knowledge has become available, making significant progress in understanding genotype to phenotype relations possible. The genes coding for these enzymes were identified, mutations in these genes in patients detected, the crystal structures of several enzymes generated and recombinant mutants expressed. In addition, recent developments in ex vivo generation of mature red blood cells from hematopoietic stem cells and insights in the assembly and cellular localisation of glycolytic enzyme complexes in the red blood cells, open new ways to investigate and understand the genotype to phenotype relations and ultimately may lead to new possibilities in treating these disorders.
Unraveling the Reactions of Nitric Oxide, Nitrite and Hemoglobin in Human Physiology and Therapeutics

Nitric oxide (NO) plays a fundamental role in maintaining normal vasomotor tone. Recent data implicate a critical function for hemoglobin and the erythrocyte in regulating the activity of NO in the vascular compartment. Intravascular hemolysis releases hemoglobin from the red blood cell into plasma (plasma cell-free plasma Hemoglobin), which is then able to scavenge endothelial derived NO 600-fold faster than erythrocytic hemoglobin, thereby disrupting NO homeostasis. This may lead to vasoconstriction, decreased blood flow, platelet activation, increased endothelin-1 expression (ET-1), and end-organ injury, and thus suggesting a novel mechanism of disease for hereditary and acquired hemolytic conditions such as sickle cell disease and cardiopulmonary bypass.

In addition to providing an NO scavenging role in the physiological regulation of NO-dependent vasodilation, hemoglobin and the erythrocyte may deliver NO as the hemoglobin deoxygenates. While this process has previously been ascribed to S-nitrosated hemoglobin, recent data from our laboratories suggest that deoxygenated hemoglobin reduces nitrite to NO and vasodilates the human circulation along the physiological oxygen gradient. This newly described role of hemoglobin as a nitrite reductase is discussed in the context of blood flow regulation, oxygen sensing, and nitrite-based therapeutics.


ADP receptors of the P2Y13 type are expressed on erythrocytes and regulate ATP release

Introduction:
Erythrocytes are involved in the regulation of blood flow and O2 delivery to tissues by release of the vasodilator ATP in response to hypoxia. ATP is rapidly degraded extracellularly to ADP by ectonucleotidases. Nucleotides like ATP and ADP activate different members of the P2 receptor family that includes 15 subtypes.

Aim of study:
We investigated if erythrocytes express ADP receptors, and if so, what functional role these receptors play.

Methods:
Real-time PCR (mRNA quantification), Western blot (P2 receptor detection), enzyme immunoassay (cAMP determination), luciferase assay following microdialysis (ATP release from incubated erythrocytes) and DNA sequencing or allele-specific PCR (SNP detection).

Results:
mRNA of the ADP receptor P2Y13 was expressed in human erythrocytes and reticulocytes, whilst other ADP receptors were not. The stable ADP analogue 2-MeSADP decreased ATP release from erythrocytes by inhibition of cAMP. The P2Y12/P2Y13 receptor antagonist AR-C67085 (30 mM), but not the P2Y1 blocker MRS2179, inhibited the effects of 2-MeSADP. At doses where AR-C67085 only blocks P2Y12 (100 nM), it had no effect. Moreover, 2-MeSADP reduced plasma ATP concentrations in an in vivo pig model. A missense polymorphism in the coding region of P2Y13 was found to be in total linkage disequilibrium with five silent P2Y12 polymorphisms previously associated with vascular disease.
Conclusion:
Our results show that erythroid cells selectively express P2Y13. ADP inhibited ATP release by acting on this receptor. This negative feedback system could be important for controlling tissue circulation and of interest for efforts to preserve intracellular ATP in erythrocytes during storage.
Regulation of PS exposure in RBC

Under normal conditions, red blood cells (RBC) exhibit an asymmetric distribution of phospho-lipids in the plasma membrane. The choline-containing lipids, phosphatidylcholine (PC) and sphingomyelin (Spm) predominantly reside in the outer leaflet, while the aminophospho-lipids, phosphatidylethanolamine (PE) and phosphatidylserine (PS), are found mainly in the inner leaflet. When PS or PE appear on the outer leaflet, the ATP-dependent aminophospho-lipid translocase (or flippase) restores the normal, asymmetric phospholipid distribution. Another transporter (MRP1) has been suggested to play a role in outward movement of phospholipids and these activities, together with as yet unknown transporters, may constitute a continuous cycle of translocation. It has been shown that increased PS exposure does occur during normal red blood cell aging in vivo, and plays a role in sequestration of senescent red blood cells.

In freshly isolated RBC, PS exposure can be detected in less than 1% of the total population by means of AnnexinV-FITC binding. Recent studies with fluorescent lipid analogues have indicated that this is due to virtual absence of outward movement of PS, indicating that in fresh RBC continuous cycling is absent. In vitro, severe loss of phospholipid asymmetry can be induced by treatment of RBC with Ca2+ and Ca2+ ionophore, by addition of phorbol ester or by exposure to singlet oxygen. All these treatments result in the activation of phospholipid scrambling and in net outward movement of PS.

During prolonged storage of RBC, scrambling activity remains low, explaining the relative minor increase in PS-positive cells during storage. However, during prolonged storage, flippase activity does decrease in parallel with the decline in cellular ATP. When stored cells are exposed to singlet oxygen (activating phospholipid scrambling), they show a higher degree of PS exposure, showing that prolonged storage does compromise RBC by affecting flippase activity.
Erythrocyte aging: aggregation, degradation, and vesiculation

In physiological circumstances, erythrocyte aging leads to binding of autologous IgG followed by recognition and removal through phagocytosis, mainly by Kupffer cells in the liver. This process is triggered by the appearance of a senescent erythrocyte-specific antigen. The functional and structural characteristics of senescent erythrocytes strongly suggest that this antigen originates on band 3, probably by calcium-induced proteolysis. Generation of vesicles enriched in denatured hemoglobin is an integral part of the erythrocyte aging process. These vesicles are also removed by Kupffer cells, with a major role for exposure of phosphatidylserine. Moreover, senescent erythrocyte-specific antigens are present on vesicles. Thus, vesicles and senescent erythrocytes may be recognized and removed through the same signals. These and other, recent data support the theory that erythrocyte aging is a form of apoptosis that is concentrated in the cell membrane, and provide the context for future studies on initiation and regulation of the erythrocyte aging process. Insight into the normal aging mechanism is essential for understanding the fate of erythrocytes in pathological circumstances and the survival of donor erythrocytes after transfusion.
Session III  Thursday 14 April, 15.45

Dorothea Stahl, W. Sibrowski
Institute for Transfusion Medicine, University of Münster, Münster, Germany
stahld@uni-muenster.de

IgM-IgG immune complexes in WAIHA

Introduction:
Warm autoimmune hemolytic anemia (WAIHA) is characterized by polyclonal IgG autoantibodies binding to red blood cells (RBC). An altered control of self-reactive IgG by autologous IgM has been proposed as the underlying mechanism of disease in WAIHA.

Aim of the study:
The current study aims at characterizing the binding properties of IgM of WAIHA patients toward normal IgG.

Methods:
IgM and IgG were purified from plasma and from RBC eluates of healthy blood donors and of patients with WAIHA using FPLC-based protocols. Binding properties of immunoglobulins were analysed under real-time conditions using optical biosensor technology.

Results:
(i) IgM in plasma of WAIHA patients exhibits an increased binding affinity toward normal IgG, as compared to the binding affinity of IgM of healthy individuals (p < 0.05).
(ii) Plasma of WAIHA patients contains a higher amount of IgM-IgG immune complexes (IC) than plasma of healthy individuals (p = 0.006).
(iii) IgM-IgG IC in plasma of WAIHA patients differ qualitatively from IgM-IgG IC in plasma of healthy individuals.
(iv) The occurrence of altered IgM-IgG IC in WAIHA is independent of the etiology of the disease.
(v) IgM-IgG IC contribute to binding of IgG to RBC in WAIHA.

Conclusion:
IgM-IgG immune complexes in plasma and associated with the RBC membrane are the characteristic feature of WAIHA. The data suggest that not the RBC itself but autologous IgG with specificity for RBC structures may be the autoantigen in WAIHA, autologous IgM being the autoantibody that leads to the formation of disease-specific IgM-IgG IC.)
Expression of phosphatidylserine (PS) on wild-type and Gerbich variant erythrocytes following glycophorin-C (GCP) ligation

Introduction:
Glycophorin-C (GPC) is a 40kDa glycoprotein on erythrocytes that is known to be used as a receptor for the malarial parasite Plasmodium falciparum to invade erythrocytes. A link between GPC and PS expression on erythrocytes has been suggested by the expression of PS on P. falciparum infected erythrocytes.

Aims of study:
PS expression has also been shown to be marker of cellular death in a number of biological pathways including erythrocyte senescence. We wish to identify red cell membrane proteins that are involved in PS exposure.

Methods and Results:
Using Annexin V binding we demonstrate that ligation of GPC with mouse mAb (BRIC-10) induces PS expression on erythrocytes. PS expression was prevented following tryptic cleavage of the extracellular domain of GPC. In addition, GPC variant phenotypes Yus (&#8710; exon2) and Gerbich (&#8710; exon 3) which express a truncated extracellular domain did not express PS following BRIC-10 binding, whereas LSa erythrocytes (that express an elongated extracellular domain) expressed PS. GPC ligation was also shown to result in a concomitant loss of erythrocyte viability in wild-type erythrocytes after 24 hours in vitro.

Conclusions:
These results identify a pathway linking GPC to PS expression on erythrocytes that may have a role in regulating cell death in erythrocytes. Further characterization of this pathway may identify new targets for the treatment of P. falciparum malaria.
Role of CD47 on RBCs as a regulator of rbc uptake and macrophage activation

CD47 (Integrin-associated protein/IAP) is a membrane glycoprotein expressed by virtually all cells in the host. The receptor for CD47 is signal regulatory protein α (SIRPα), which is expressed by leukocytes, neuronal cells and endothelial cells. When expressed by leukocytes (monocytes, macrophages, and dendritic cells) SIRPα functions as an inhibitory receptor. We have found that unopsonized red blood cells (RBCs) from CD47-deficient (CD47-/-) mice are rapidly cleared from the circulation in CD47 wt mice (1). This RBC clearance is not mediated by antibodies, lymphocytes or complement, as clearance of CD47-/- RBCs was also seen in Rag1-/- and C3-/- CD47 wt recipients. Instead, CD47-/- RBCs were shown to be specifically eliminated by splenic red pulp macrophages due to the absence of inhibitory CD47/SIRPα interaction both in vivo and in vitro. CD47-/- lymphohematopoietic cells (LHC) were also eliminated by the same mechanism in CD47 wt recipients (2). For clearance of CD47-/- LHC, however, both macrophages and dendritic cells were involved. Thus, macrophages and dendritic cells have a mechanism for self vs. non-self discrimination, similar to the established “missing self hypothesis” described for NK cells, but where the marker of self recognized is not MHC class I, but rather CD47. The activating receptor on macrophages/dendritic cells, and its ligand on unopsonized circulating blood cells, in this system are not known. We next showed that inhibitory CD47/SIRPα signalling can also regulate Fcγ and complement receptor-mediated phagocytosis of opsonized RBCs in macrophages, both in vivo and in vitro. IgG or C3b-opsonized CD47-/- RBCs were phagocytosed to a greater extent than CD47 wt RBCs opsonized at the same level (3). The inhibition generated by CD47-SIRPα interaction is strongly attenuated but not absent in mice with only residual activity of the phosphatase SHP-1, suggesting that most SIRPα signaling in this system is mediated by SHP-1 phosphatase activity. The macrophage phagocytic response is controlled by an integration of the inhibitory SIRPα signal with pro-phagocytic signals such as from Fcγ and complement receptor activation. We also recently showed that macrophage uptake of IgG-opsonized platelets is regulated by CD47/SIRPα -interaction in the same way (4). It is known that NOD mice that do not die from diabetes can develop a mild form of AIHA at an older age (>1 year). Interestingly, CD47-/- NOD mice that do not develop diabetes all
succumb from spontaneous acute lethal AIHA before 280 days of age, and CD47-/- C57BL/6 mice also showed acute sensitivity to experimental AIHA induced by a pathogenic anti-RBC antibody (5). In conclusion, by interacting with the inhibitory receptor SIRPα, CD47 can regulate macrophage uptake of RBCs and other blood cells.

CD47 – an inducer of RBC apoptosis

Introduction:
CD47 is a 47-50 kDa membrane glycoprotein with 5 known isoforms. The functional role of CD47 within the erythrocyte membrane remains the subject of much research and debate with both pro and anti apoptotic functions assigned. A number of extracellular CD47 ligands have been suggested and three studies have provided indirect evidence that CD47 binds to protein 4.2 of the RBC membrane skeleton.

Aims of Study:
CD47 is known to induce Caspase-Independent Apoptosis (CIA) in a number of cells. We wish to study the expression of CD47 and identify the molecular binding partner(s) of each isoform. We hypothesise that CD47 may be an inducer of CIA in erythroid cells.

Methods and Results:
We demonstrate ubiquitous expression of all five CD47 isoforms in haemopoietic cells and tissues using RT and QRT PCR. Yeast II Hybrid analysis has further resolved interactions of CD47 and Co-Immunoprecipitation / de-novo sequencing has identified a novel ternary complex involving CD47/p4.1R/Actin/p55. We also demonstrate using Annexin V FITC binding that CD47 mAb ligation induces PS exposure and a concomitant loss of cell viability in mature erythrocytes.

Conclusions:
We provide evidence for a role of CD47 in erythrocyte apoptosis. We propose p4.1R/p55 links CD47 to the apoptotic machinery of the cell and suggest a mechanism whereby cytoskeletal rearrangement and PS exposure occurs, possibly following CD47 ligand binding. This work may provide methods to enhance RBC survival during storage.
The sequence of events leading to storage of blood components

Heterologous blood transfusion leads to disaster. Therefore, transfusion was restricted to homologous blood due to, amongst others, ABO-Rhesus D blood group antigens on the blood cells. Transfusion required blood group typing of donors beforehand.

Blood collection was made possible by the invention of a sterile blood taking set, consisting of a vena puncture needle, a collection vessel and a connection tube. Whole blood preservation outside the human body was possible after discovery of the anticoagulant citrate. Direct transfusion became possible after the invention of a transfusion set that allowed anticoagulation during the transfusion of blood with the aid of a three-way-cock.

Storage of blood prior to transfusion facilitated medication – and blood collection – logistics by allowing blood banking. Sterile storage of whole blood required a container prefilled with a mixture of anticoagulant and nutrient. This container became a glass bottle with a rubber closure, that could be sterilized. The rubber closure resealed scars of needle punctures. Sharpening of the needle point by double facet grinding and blunting the heel of its aperture prevented punching during puncture. An acid mixture of citrate and glucose (ACD) allowed sterilization without caramel formation.

Each blood component required specific storage conditions. Storage of whole blood leads to aggregation, haemolysis and proteolysis. Leukocyte contamination caused immunological transfusion reactions. Rigid bottles required a vent to allow in- and outward transfer of liquid. Puncture scars in the rubber closure opened in reaction upon internal pressure changes during cold storage of bottled blood. Bacterial contamination and growth during storage was unavoidable.

Development of collapsible blood bags allowed sterile separation in a closed system by centrifugation, based on the density of components and the size of cells. Bag centrifuges, component expressors and sterile docking of tubes were invented as well.

Leukocyte removal by selective filtration prevented immunization and transmission of germs in transfusion.

Plasma constituents were preserved by rapid freezing and storage below the eutectic point (minus 23°C). Red blood cells were stored at 4°C in a nutrient medium that preserved the levels of ATP and 2,3 BPG by anaerobic glycolysis.

Platelets had to be prepared at temperatures >20°C and stored at 22°C, in an aerated gas permeable bag, suspended in a nutrient...
medium that allows the preservation of their morphology and ATP content by aerobic glycolysis.

The threat of germ transmission by transfusion led to new selection criteria for donors, donation safety precautions, traceability of blood components, new blood tests and digitalisation of testing and administration.

Blood components became products manufactured by pharmaceutical standards. Economy of scale and logistics required industrialization and automation of donor registration, donor call, donation registration, component separation, product release procedures, and medication registration.

These requirements implicate every change in component preparation and storage. Moreover the consequences of any change have to match with the convenience requirements of the donation. The solution of bedside component preparation is no option, as it throws the level of donation convenience and logistics back to the pre-blood-banking era of direct transfusion.
Basic scientific advances and hypothesis-driven experiments have led to better understanding of the red blood cell (RBC) storage lesion and to ways to improve RBC storage solutions and storage systems. The most important scientific advance has been the recognition that the storage lesion is a form of programmed cell death that can be held in check by maintaining the intra-cellular concentration of adenosine 5’-triphosphate (ATP) near normal. Normal concentrations of ATP maintain phospholipid asymmetry, prevent the evolution of membrane microvesicles, and reduce hemolysis. RBC ATP concentrations can be kept in the normal range during storage by providing appropriate concentrations of adenine, phosphate and glucose and by maintaining the storage system in the narrow pH range of 7.2 to 6.5. Keeping RBC in this narrow pH range by starting with alkaline additive solutions which use bicarbonate for buffering allows RBC to be stored for at least 8 weeks with better 24 hr in vivo 51Cr recovery, lower hemolysis, and less membrane loss than conventional saline-adenine-glucose-mannitol additive solutions achieve with 5-6 weeks of storage. These new alkaline additive solution systems are in commercial production and are undergoing human testing preliminary to licensure. The use of such systems will mean that all patients receiving stored RBC will receive cells with better flow characteristics and a lower burden of non-viable cells.
Dirk de Korte  
Dept. of Blood Cell Research, Sanquin Research, Amsterdam, The Netherlands  
d.dekorte@sanquin.nl

Pathogen reduction in RBC preparations

For cellular blood products, safety relies on selection of donors and screening of blood donations, because methods for pathogen inactivation (PI) as used in the production of plasma derivatives cannot be applied. Several PI technologies for Red Blood Cell (RBC) preparation are under development and some of them were close to clinical application. The major developments will be discussed in this presentation.

Various groups are still developing photochemical methods, despite the problem that for homogenous, effective illumination, it is necessary to dilute the RBC, due to the quenching by hemoglobin. Therefore, the final product is not suited for transfusion. To solve this problem, several groups are combining a photochemical method with washing and concentration steps. The reported results with photochemical approaches are promising with respect to pathogen killing, but in most cases there is a significant effect on the in vitro quality of RBC, with increased hemolysis and other signs of decreased quality at the end of storage.

Other groups changed to non-photochemical methods, as did Cerus with their compound S303 and Vitex, with their compound Inactin. These compounds are added to the RBC and during the subsequent incubation the pathogens are killed by intercalation of the compound with DNA/RNA. Both companies reported very effective killing for viruses, bacteria and parasites, with limited negative effects on the in vitro quality of RBC. In contrast to the situation for platelets, the reported effects on in vitro quality did not influence the storage time of the RBC and both companies were successful in starting clinical trials. However, recently, both phase III clinical trials, with respectively S303 and Inactin treated RBC, had to be halted due to immunological responses on the treated erythrocytes.
Iron Metabolism in red blood cells

Background:
The main driving force of iron metabolism is haemoglobin synthesis in erythroblasts. A defect in this process may produce anaemia, which is either associated with iron deficiency or with iron overload.

Review of the subject:
Mitochondria play a crucial role in iron kinetics as they are the site of haem synthesis. The impact of changes in Hb synthesis on iron absorption and iron storage will be explained, as well as the function of iron transport molecules.

A wide variety of clinical disorders arises from genetic and acquired defects of porphyrin and haem synthesis. This process starts in the mitochondrion, travels towards the cytosol, and returns to the mitochondrion for final insertion of iron into protoporphyrin to form haem.

By far the most common mitochondrial iron disorder is simple iron deficiency anaemia. Due to an imbalance between iron and available protoporphyrin insufficient amounts of haemoglobin are produced to fill up erythrocytes, resulting in hypochromic anaemia. The cell tries to compensate this by upregulating expression of the enzyme ALA-synthase (regulated at the mRNA level by Iron Responsive Protein) resulting in an increased production of porphyrins. Many enzymes are involved in the chain of haem synthesis. A genetic defect of ALA-synthase causes sideroblastic anaemia (there are, however, also acquired forms), resulting in more or less severe dyserythropoietic anaemia and progressive secondary iron overload. In bone marrow aspirates typical “sideroblasts” can be recognized showing accumulation of iron in mitochondria.

Enzyme defects more downstream the chain of porphyrins can cause a variety of clinical disorders, called porphyrias, which are remarkably not associated with anaemia but with several forms of dermatological, neurological, psychiatric or gastro-intestinal symptoms. Some of these disorders are associated with (mild) iron overload and others with iron deficiency. Pathology is mainly due to toxicity of accumulating porphyrin precursors.
Many other RBC defects interfere with iron metabolism. These include thalassemias and haemoglobinopathies, defects of the cytoskeleton and the glycolytic pathway, in particular those resulting in haemolysis, dyserythropoiesis and frequent erythrocyte transfusion.

Changes of iron metabolism in red cell disorders will be demonstrated using results from radioiron measurements of iron absorption and plasma ferrokinetics, together with more common iron parameters.
The penetrance of hereditary hemochromatosis: controlled biochemical and clinical observations in a normal population

The body conserves its stores of iron tenaciously to avoid iron deficiency. It maintains iron homeostasis by regulating gastrointestinal absorption; there is no pathway for iron excretion. Excess iron may accumulated in the body when the regulation of iron homeostasis breaks down either by the introduction of excessive iron into the body, usually in the form of transfusions, or as a result of a genetic defect in one of the enzymes of iron homeostasis. Excessive iron may produce cirrhosis of the liver, diabetes, darkening of the skin, hypogonadism, and cardiomyopathies, the syndrome of hemochromatosis.

In recent years it has been shown that hemochromatosis can occur as a result of mutations in the HFE, transferrin receptor-2, hepcidin, ferroportin, and hemojuvelin genes. The most common are those of the HFE gene, which reach polymorphic frequencies. Even before the HFE gene was cloned, it was recognized that it was very common in the population, and it could be estimated that about 5 per 1,000 people of northern European ancestry are homozygous. It was believed, at one time, that many or most of these individuals developed symptoms as a result of iron overload, but without a controlled study the penetrance of the homozygous state could not be properly evaluated. The symptoms that were ascribed to hemochromatosis included such common afflictions as fatigue, arthritis, impotence, and diabetes, conditions that all have a fairly high prevalence in the middle-aged and older population.

We had the opportunity to study the penetrance of the HFE mutations in a well-controlled study at Kaiser Permanente in San Diego, CA, USA and found that less than 1% of patients homozygous for the mutation had the full-blown clinical syndrome of hemochromatosis. Another 10 to 20% of the homozygotes manifested abnormalities in one or another liver function test. However, there was no significant effect on the lifespan and no difference was found between the actual clinical manifestations of the 99+% of homozygotes and matched individuals without HFE mutations. The reason why only rare individuals who are homozygous for hereditary hemochromatosis develop significant
clinical manifestations is of major importance. We have studied approximately 30 candidate genes in individuals with high penetrance, thus far, without finding a common explanation for the high penetrance of hemochromatosis in only a few individuals.
Hemoglobin-based Red Blood Cell substitutes

Safe and effective alternatives to red blood cells for transfusion have been sought for decades\(^1\). In principle, such solutions would alleviate the need to type and crossmatch blood, have prolonged shelf life and could be used immediately in cases of trauma or emergency. Ideally, they would also be inexpensive and readily available. Haemoglobin, the oxygen carrier of red cells, is itself not suited to be administered as a cell-free solution because outside of the red cell the protein rapidly dissociates into monomeric subunits, iron oxidizes, and toxicity in the form of vasoconstriction and tissue damage result. Chemical modification of the protein has been extensively explored, and it is possible to produce derivatized hemoglobin of nearly any size and configuration with prolonged intravascular circulation and stability. While some of these molecules have been tested in human trials, the mechanisms that mediate their toxic effects have not been well understood, and many of the trials have been unsuccessful.

Maleimide-PEG modified human hemoglobin (MP4)\(^5\) that is not vasoactive in the hamster skinfold model and releases O\(_2\) specifically in hypoxic tissue\(^6\) incorporates the lessons learned from these studies and has shown promise in several animal models of hemorrhagic shock and hemodilution. MP4 is now progressing through human clinical trials. It is anticipated that new products that are designed with local tissue needs and circulatory characteristics in mind will be more successful than ones designed to merely duplicate the O\(_2\) transport properties of red blood cells. Such
products, capable of delivering $O_2$ effectively and safely to tissues, may not be “red cell substitutes” in every sense, but may very effectively serve the function of red cells for which they are transfused: tissue oxygenation.


The ability of blood and its alternatives to oxygenate the microcirculation

Blood transfusion is expected to improve tissue oxygenation during anemia and shock by its capacity to transport oxygen. During storage however this capacity of blood seems to be affected and there is discussion about the determinants of stored red blood cells which need to be preserved to maintain this quality of red blood cells. To evaluate the conditions under which blood transfusions are effective carriers of oxygen, however, requires insight into the determinants of microcirculatory and cellular oxygen delivery during conditions of anemia, ischemia and hypoxemia and resuscitation. To investigate these issues we developed and applied the Pd-porphyrin quenching of phosphorescence technique to quantitatively measure microvascular pO\(_2\) (\(\mu\)pO\(_2\)) \textit{in vivo}\(^{1,2}\). We found consistently that during hemorrhage, septic shock or reperfusion injury, \(\mu\)pO\(_2\) fell to values below that of the mesenteric venous pO\(_2\), indicating that the microcirculation was being shunted, creating a pO\(_2\) gap\(^{3,4}\). Resuscitation with stored red blood cells may correct this pO\(_2\) gap seen in hemorrhage, and excessive storage may affect the ability of red blood cells to transport oxygen effectively. We investigated this issue in rats and found that fresh stored rat blood was indeed able to restore gut \(\mu\)pO\(_2\) and venous pO\(_2\) values back to base-line values, whereas 28-day stored blood was not able to restore gastric \(\mu\)pO\(_2\) \(^{5}\).

One of the properties of red blood cells which might effect its ability to transport oxygen to the microcirculation is its ability to deform and enter the capillary bed. Due to the rapid aging of rat blood cells we subsequently developed a rat model able to tolerate the transfusion of stored human red blood cells. In this model we found that human red blood cells stored longer than 3 weeks lost their capacity to transport oxygen to the gut microcirculation\(^{13}\).

Alternatives for homologous blood transfusions include hemodilution (HD) and the use of haemoglobin-based oxygen carriers (HBOC). In HD experiments in pigs and rats, we showed that \(\mu\)pO\(_2\) is maintained during hemodilution despite a decreasing venous pO\(_2\) \(^{8}\). In investigating the ability of HBOCs to improve gut microvascular oxygenation in pigs we showed that small volumes of HBOC were effective in resuscitating gut \(\mu\)pO\(_2\) and pO\(_2\) gap following hemorrhage\(^{10}\). In subsequent studies we investigated the effect of different volumes of DCLHb in heart and gut microcirculatory oxygenation simultaneously\(^{11}\) as well as recently the use of recombinant Hb\(^{14}\).
References
Blood substitutes for clinical use

Russia is the only country in the world where blood substitutes – oxygen carriers (BSOC) of both classes – polymerized hemoglobin solution (preparation Gelenpol) and perfluorocarbons (PFC) emulsion (preparation Perftoran) have been registered and approved for clinical use. These preparations are used as temporary oxygen support during large operations in cardiopulmonary, trauma, orthopedic and abdominal surgery; for emergency resuscitation after traumatic blood loss; as short-term red blood cell support in chronic anemia and other conditions; and in anemic Jehovah’s Witness patients whose religious beliefs do not allow them to use donor blood.

Our clinical experience with the use of Perftoran (with the high inspired oxygen concentration) and Gelenpol for blood loss resuscitation in acute gastro-duodenal hemorrhages and in hemorrhagic and traumatic shock has shown that both BSOC increase oxygen capacity of mixed venous blood, ensure oxygen delivery to tissues, decrease the need for donor blood or blood components in perioperative periods with a factor 2. Perftoran proved to be more efficient with respect to rheology of blood in the microcirculation while Gelenpol is better in stabilization of hemodynamics and erythropoiesis stimulation. It should help in infusion-transfusion program development to combine both BSOC. Perftoran (perfluorocarbons emulsion) is included in the compulsory set of transfusion medicines in the Russian army, Gelenpol (polymerized hemoglobin solution) is on the way to large-scale production.
Session VII  Friday 15 April, 13.00

Timothy. C. Fisher¹, J.K. Armstrong¹, R. Leger², P. Arndt², G. Garratty²

¹. Keck School of Medicine, USC, Los Angeles, California, USA
². American Red Cross Blood Services, Los Angeles, California, USA
tcfisher@usc.edu

The Current Status of PEGylated Red Blood Cells

Attachment of poly(ethylene glycol) (PEG) to the surface of red blood cells (RBCs) can prevent blood group antigens from being recognized by antibodies, which may be useful for blood transfusion. Using crosslinked PEG coatings it is possible to mask all blood group antigens such that they are not detected when tested by standard serologic methods (including IAGT) and immunoglobulin-mediated adherence/phagocytosis is suppressed in a monocyte monolayer assay. However, at present, the types of PEG coating that can fully mask human RBC antigens causes accelerated RBC clearance in a rabbit model (using rabbit RBCs). “Lighter” coatings containing less PEG result in normal RBC survival in the rabbit model, but do not provide complete masking when applied to human RBCs. Such coatings may still be useful however, since many minor antigens are masked, and also because the coating reduces blood viscosity.

“PEGylation” of proteins such as enzymes and cytokines to reduce antigenicity and improve circulation times has become common practice, and it is generally assumed that PEG itself is a non-antigenic and non-immunogenic molecule. However, plasma samples from many normal donors will directly agglutinate autologous or compatible PEG-coated RBCs. To further investigate this phenomenon, we studied plasma samples from 250 normal blood donors. Of these, 62 (25%) directly agglutinated fully-compatible PEG-RBCs via tube test (IS); 12 samples (5%) caused 3+ or 4+ agglutination. The serology results were confirmed by flow cytometry using PEG-bearing “Tentagel” beads: 63 samples (25%) were positive; 46 samples (18%) showed IgG binding only, 9 (4%) contained IgM only, and 8 (3%) contained both IgG and IgM. Almost all IgGs subtyped as IgG2. The 25% prevalence of a PEG-reactive antibody in normal human subjects is much greater than expected. For comparison, a 1984 study in Sweden found 1 in 500 normal sera reactive with PEG-RBCs. (Richter AW, Akerblom E, Int Arch Allergy Appl Immunol 74(1):36-9)

Complete inhibition of agglutination was achieved by addition to the plasma of 1% w/v of small PEG molecules (300 Da), tri- and tetra-(ethylene glycol) dimethyl ether and penta(ethylene glycol). Di- and tri-(ethylene glycol) had no effect, and neither did irrelevant polymers such as dextran and polyvinyl alcohol. These results appear to confirm that the antibody has true anti-PEG specificity, and suggest that it recognizes a minimum epitope equivalent to
about four monomer units. The IgG anti-PEG has been isolated in low yield from human plasma by affinity purification. Studies in rabbits indicate that PEG can be significantly immunogenic, at least when bound to RBCs. Twelve of 17 rabbits receiving PEG-RBCs developed an antibody to PEG. This was detected after the first infusion of PEG-RBCs in 6 rabbits, after 2 infusions in 4 rabbits, and after 3 or more infusions in 2 rabbits. Two of the animals showed a sustained (>1 year) antibody response (3+ and 2+ by direct agglutination); the other 10 showed a variable response that could be was boosted by repeat challenge with PEG-RBCs. Of the 5 non-responders, 4 received 3 infusions, and one 7 infusions. Clearance of PEG-RBCs was accelerated after the development of anti-PEG.

In conclusion, it is apparent that PEG can be both antigenic and immunogenic, and while PEG-RBCs may be useful for specific problems in transfusion, such as for multiply alloimmunized patients, or to protect donor or host RBCs in AIHA, it seems unlikely that PEG-coating will have broader application, unless the anti-PEG in humans can be definitively shown to have minimal practical significance.
Enzyme-conversion of red blood cells for ABO-universal transfusion

Eliminating the risk for ABO-incompatible transfusion errors and simplifying blood logistics by creating an ABO-universal blood inventory is a challenging idea but not a new one. In the early 1980s Goldstein and co-workers at the New York Blood Center pioneered the field of enzymatic conversion of blood group B red blood cells (RBC) to group O (Science 1982;215;168-70). Originally, α-galactosidase from coffee beans was used to remove the immunodominant galactose residues terminally located in the carbohydrate chains found on group B RBC. Later they showed that recombinant enzyme worked equally well. These enzyme-converted O (ECO) RBC survived normally in the circulation of individuals independent of the blood group of the recipient as measured by haemoglobin increments and RBC survivals based on 51Cr-labelling studies. Small infusions were escalated to full RBC units, multiple units and repeated transfusions. A successful phase II clinical trial was reported in 2000 by Kruskall et al. (Transfusion 2000;40:1290-8).

Conversion of blood group A cells is a much more complex task that was never accomplished during the early studies. This was mainly due to lack of the appropriate enzyme, α-N-acetylgalactosaminidase, working at RBC-friendly conditions. Also, the chemical nature of group A antigens is more complex due to the A1/A2 subgroups and the presence of A type 3 (the repetitive A epitope, mainly in glycolipids of the A1 subgroup) in addition to the simpler A type 1 and 2 chains. However, the recent identification of novel bacterial exoglycosidases with improved kinetic properties and specificities for the blood group A and B antigens has revitalized the field significantly. ZymeQuest, Inc. (Beverly, MA, USA) surveyed >2000 bacterial and fungal isolates for A-zyme and B-zyme activities, and discovered both A- and B-degrading enzymes (A-zymes and B-zymes) with much improved characteristics compared to previously reported exoglycosidases. The selection criteria included high specific activity, optimal activity at neutral pH, and strict specificity for the blood group A and B structures. Numerous enzymes were cloned, expressed and tested to assess their efficiency in ECO conversion. The chosen A- and B-zymes are members of a novel gene family, the original function of which may be to act as virulence factors in pathogens trying to invade hosts in populations with diverse blood groups. Enzymatic conversion of all non-O RBC can now be achieved with these improved glycosidases. In fact, both A-ECO and B-ECO RBC
type as native group O RBC with all available monoclonal blood grouping reagents. No other blood group antigens are modified in the process. In addition, the conversion conditions for both blood group A and B have been optimized to use neutral pH and short incubation time at room temperature as well as cost-efficient quantities of recombinant enzymes. Development of single- and eight-unit converting devices is ongoing and technological platforms supporting large-scale enzyme conversion are available for study purposes.

Of the different strategies envisioned to create a universal blood component supply, the ECO concept is the only one for which human clinical trials have been performed. The presentation will review the current status of this interesting technology and its potential for introduction in the clinical component preparation laboratory.

The author is a Visiting Associate Professor at Harvard Medical School and also a scientific consultant to ZymeQuest, Inc., Beverly, MA, USA. He gratefully acknowledges the significant and dedicated work performed by numerous collaborators at Copenhagen University, Harvard Medical School, Lund University and most importantly the scientific staff at ZymeQuest, Inc.
Heme Oxygenase-1: A Potential Modulator Of Inflammation And Vaso-Occlusion In Sickle Cell Disease

Hemolysis in sickle cell patients leads to elevated plasma hemoglobin S and bilirubin, increased numbers of circulating reticulocytes, reduced plasma haptoglobin and hemopexin, increased kidney and endothelial cell HO-1 expression and carbon monoxide (CO) production. Plasma hemoglobin S can transfer heme to endothelium thereby enhancing the production of reactive oxygen species (ROS), activating vascular endothelium and inducing the cytoprotective enzyme heme oxygenase-1 (HO-1). We hypothesize that HO-1, an adaptive, anti-inflammatory gene, and its downstream products, play a vital role in the inhibition and resolution of vaso-occlusion in sickle cell disease. S+S Antilles transgenic sickle mice have an activated vascular endothelium, that includes enhanced nuclear factor kappa B (NF-κB) activation and endothelial cell adhesion molecule (ECAM) expression. These transgenic sickle mice also hemolyze in vivo as evidenced by increased reticulocyte counts (10.2%), plasma hemoglobin (2.3 mg/dl) and bilirubin (1.7 mg/dl) and reduced plasma haptoglobin compared to normal control mice (p<0.01). HO-1 expression was increased in the lungs (5.8-fold), kidneys (6.5-fold) and spleens (3.4-fold) of sickle mice compared to normal mice (p<0.05). Treatment of sickle mice with hemin (40 μmoles/kg i.p./d x 3) further increased HO-1 expression in the lung, liver, spleen and kidney. HO-1 upregulation by hemin treatment decreased lung, liver and kidney NF-κB over-expression in sickle mice by 56-80% (p<0.05). VCAM-1 and ICAM-1 over-expression in sickle mouse livers was reduced by 50% and 70% respectively, after hemin treatment (p<0.05). Treatment of sickle mice with an HO-1 inhibitor, tin protoporphyrin, had no effect on liver VCAM-1 expression, but increased liver ICAM-1 expression by 75% (p<0.05). Treatment of sickle mice with the HO-1 gaseous enzymatic product, carbon monoxide (CO, inhaled at 250 ppm for 1h for 3d) diminished NF-κB activation in the liver by 70% and the kidney by 40% (p<0.05). Leukocyte rolling and adhesion in the dorsal skin venules of sickle mice were inhibited by 70% (p<0.01) after global HO-1 upregulation by hemin. In response to hypoxia/reoxygenation, blood flow in venules of the dorsal skin became
Upregulation of HO-1 by hemin, or treatment with HO-1 products biliverdin (50 μmoles/kg/d x 3) or CO prevented stasis (p<0.05), while HO-1 inhibition by Sn-protoporphyrin significantly prolonged and worsened vaso-occlusion (p<0.05). We hypothesize that HO-1 modulates vaso-occlusion through multiple mechanisms including NF-κB inhibition, endothelial cell adhesion molecule down-regulation, decreased RBC hemolysis and altered vascular tone. In sickle cell disease patients, adaptation of HO-1 activity may be cytoprotective, suggesting that normal levels of HO-1 are inadequate to handle the excessive heme burden. We believe that further upregulation of HO-1 activity and/or its downstream products will be important strategies for preventing and treating vaso-occlusion in sickle cell disease.
Hematopoietic stem cell transplantation in sickle cell anemia

In a prospective study of the Cooperative Study of Sickle Cell Disease based on 3764 patients, it was shown that life expectancy in patients suffering from sickle cell disease was decreased by 25 to 30 years, as compared with the black American population in general. Preventive and supportive measures were the main therapeutic tools available until the last decade. Hydroxyurea has been shown to improve the clinical course in both adults and children. However, hematopoietic stem cell transplantation remains the definitive treatment for cure and eradication of the disease. In our cohort of patients, the overall survival and the disease free survival are 93% and 85% respectively, reaching 100% and 93% if we consider only young patients transplanted before the occurrence of symptoms and thus before chronic organ damage. So far, almost 200 patients have been transplanted for sickle cell anemia worldwide. Selection criteria have to put into balance the chances of cure against toxicity and long term side effects of stem cell transplantation.

Vermylen C., Fernandez Robles E., Ninane J., Cornu G.


Locatelli F., Rocha V., Reed W., Bernaudin F., Ertem M., Grafakos S.,
Brichard B., Li Xx., Nagler A., Giorgiani G., Haut Pr., Brochstein Ja.,
Nugent Dj., Blatt J., Woodard P., Kurtzberg J., Rubin Cm., Miniero R.,
Lutz P., Raja T., Roberts I., Will Am., Yaniv I., Vermyleen C., Tannoia
N., Garnier F., Ionescu I., Walters Mc., Lubin Bh., Gluckman E.
Related umbilical cord blood transplantation in patients with
Blood-Saving Techniques in Cardiac Surgery

Large amounts of transfusions are common in cardiac surgery. Therefore intensive support of blood banks is mandatory for successful cardiac surgery. In this presentation the different reasons for increased blood loss and transfusion in cardiac surgery compared to other surgical specialties will be discussed with emphasis on pharmacological and technical solutions for improvement.
Recombinant human erythropoietin (rHuEpo) therapy has primarily been developed for the treatment of anemias associated with Epo deficiency. rHuEpo is thus very efficient for the treatment of the anemia of chronic renal failure, but higher doses are required in other forms of anemia, including the anemia of cancer, chronic inflammatory disorders, HIV infection or prematurity. rHuEpo also has remarkable efficacy after autologous as well as allogeneic hematopoietic stem cell transplantation, provided it is started after engraftment. rHuEpo has also been used for the prevention of anemia in patients without Epo deficiency, such as patients scheduled for surgery and/or undergoing a program of autologous blood donation.

The benefits procured by rHuEpo therapy include increased hemoglobin levels, decreased transfusion requirements, improved quality of life and work capacity. Animal studies also suggest that rHuEpo therapy may improve cancer response to chemotherapy or radiotherapy and possibly increase survival. However, two clinical studies in cancer patients undergoing chemotherapy or radiotherapy were stopped prematurely because of poorer outcome in patients receiving rHuEpo. Other, better-designed clinical trials are under way to examine whether rHuEpo therapy can indeed improve survival in cancer patients.
There are a number of limitations to the efficacy of rHuEpo therapy, i.e. infections, inflammation, surgery, bleeding, hemolysis, limited residual hematopoiesis, anti-Epo antibodies, and particularly (functional) iron deficiency. Measures destined to improve the cost/benefit ratio of rHuEpo therapy involve SC rather than IV administration, weekly rather than thrice weekly injections, adequate iron supply in the form of IV iron, newer (long-acting) forms of rHuEpo, combination with other growth factors, adequate treatment for intercurrent causes of resistance, and better patient selection based among other things on predictors of response.
Session IX  Friday 15 April, 17.05

Cynthia So-Osman¹, R.G.H.H. Nelissen², A. Brand¹
¹. Sanquin Bloodbank Southwest Region, Leiden, The Netherlands,
². Dept. of Orthop. Surg., LUMC, Leiden, The Netherlands
cynthia.so-osman@bloodrtd.nl

Post-operative re-infusion of shed blood

Introduction:
Postoperative reinfusion of shed red blood cells (RBC) may not only save the use of allogeneic RBC transfusions, but preliminary studies also suggest a decrease in postoperative infections.

Aim of the study
To determine the efficacy and safety of two different postoperative autologous blood reinfusion systems (Bellovac ABT-Astra Tech- and DONOR- Van Straten Medical-) a randomized, controlled study is carried out in elective orthopaedic patients in the Leiden University Medical Center (LUMC).

Patients and methods:
From 2001-2003 consecutive primary and revision total hip-(THR) and knee replacement (TKR) patients from 18 years of age were randomized for :
a/ control group without postoperative reinfusion of shed blood
b/ postoperative reinfusion of shed blood by DONOR-system
c/ postoperative reinfusion of shed blood by Bellovac-ABT system
By means of a questionnaire the nursing staff scored efficacy. Regarding safety, patients were monitored after reinfusion.

Results
69 of 70 patients were evaluable. Efficacy of both reinfusion systems were comparable.
In 20% (6/30) a transient (mostly febrile) transfusion reaction was seen. No other adverse reactions were seen. Surprisingly, in multivariate analysis, hospital stay (divided into two groups: < 8 days vs. 8 days or more) was significant lower in the reinfusion groups than in the control group.

Conclusions:
both postoperative autologous blood reinfusion systems were feasible and safe in use. We found an interesting difference in hospital stay. Further studies are needed to investigate the clinical effect of shed blood.
List of participating companies – medical technical exhibition

1. Baxter
   Kobaltweg 49
   3542 CE Utrecht
   The Netherlands

2. Fresenius Hemocare
   Runde ZZ 41
   7881 HM Emmer Compascuum
   The Netherlands

3. HemoCue Nederland BV
   P.O. Box 319
   5060 AH Oisterwijk
   The Netherlands

4. Sanquin Blood Supply Foundation
   Sanquin Reagents
   P.O. Box 9892
   1006 AN Amsterdam
   The Netherlands

5. Haemonetics BV
   Tinstraat 107
   4823 AA Breda
   The Netherlands

Social programme

Drinks and Congress dinner Thursday April 14th

The drinks and dinner will be held on board of canal boats. The boats will depart at 18.30 hours opposite of the Rode Hoed. Accompanying persons are requested to assemble in the lobby of De Rode Hoed between 18.15 and 18.30 hours.

Price: € 35.-

If you have not booked already, our staff at the registration desk will be happy to inform you if seats are still available.

Farewell drinks

On Friday, after the last session a farewell reception will be organised in De Rode Hoed. You are most welcome to join this reception.
General information (in alphabetical order)

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

Banking Facilities
The official currency in The Netherlands is the Euro (€). It is recommended that foreign currencies will be converted to Euros at Dutch chartered banks, which are usually open from Monday through Friday from 09.00 – 16.00 hours. Exchange of foreign money and travellers’ cheques is also possible in most hotels.

Cloakroom and Luggage
In De Rode Hoed a cloakroom is located near the registration area. Luggage may be taken on board during the social event.

Electricity
In the Netherlands, electricity is supplied at 220 V – 50 Hz AC.

Insurance
In registering for the Sanquin Spring Seminars, participants agree that neither the Organizing Committee nor the Seminar Secretariat assume any liability whatsoever. Participants are requested to make their own arrangements for health and travel insurance.

Language
The official language of the Sanquin Spring Seminars is English.

Registration Desk
The registration desk will be open at the following times:
Thursday, April 14, 2005  09:00 – 18:30 hours
Friday, April 15, 2005  08:00 – 17:30 hours

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

Taxis
Numerous taxi stands are located throughout Amsterdam. The telephone number of the central taxi service is 020-7777777.

Weather
While April may offer lovely spring weather, as can be seen in the printings of the old Dutch masters, it can be quite unpredictable and might be chilly in the evening. Temperatures range from 7 to 14°C. As showers might occur, we advise you to bring a raincoat or an umbrella.
COMPOMATION™ DATA MANAGEMENT SYSTEM

CompoGuard® and Donationmaster™

Bi-directional interface to blood bank host

Compomat® G4 and Compomaster™
The next Sanquin Spring Seminar will again be held in Amsterdam, in the spring of 2007, Thursday 19 and Friday 20 April 2007, Amsterdam, The Netherlands

Antibodies in Disease, Diagnosis and Treatment

Topics to be discussed will be:
- The repertoire of natural antibodies
- Artificial antibodies in diagnostics
- Antibodies against pharmaceuticals
- Antibodies, a new class of drugs
- Antibodies against leukaemia
- Antibodies as drug carriers
- Recombinant anti-D prophylaxis
- The mechanism of action of IVIG