ISSN 0009-9120
Volume 42
Number 18
December 2009

CLINICAL BIOCHEMISTRY

continued on back cover

INDEXED/ABSTRACTED IN: Current Contents/Life Sciences, Index Medicus, MEDLINE, BIOSIS Database, Chem Abstracts, Current Awareness in Biological Sciences (CABS), Reference Update. Also covered in the abstract and citation database SCOPUS®. Full text available on ScienceDirect®.

Available online at www.sciencedirect.com
ScienceDirect
Clinical Biochemistry 42 (2009) 1851 – 1863
CLINICAL BIOCHEMISTRY

Abstracts—Oral Communications

17th International Symposium of the European Association for Red Cell Research, EARCR 2009, Triuggio, Milano, Italy, April 23–27, 2009

OPENING LECTURE

Chloride conductance of the normal human red cell – past and present

Poul Bennekou, Biological Institute, University of Copenhagen, Copenhagen, Denmark

The picture of human erythrocyte chloride transport started emerging 50 years ago. It was shown to incorporate a fast exchange and a slower conductive component, and both were inferred to be mediated by the same protein, (band 3/AE1/SLC4A1), with $\sim 10^6$ copies present in the membrane. Two extremes can be imagined: the conductive transport is maintained all the time by all copies in some sort of carrier mode, or a few at a time enter a conductive mode, become "channel like."

Using patch clamp, more and more anion channels have been demonstrated in the normal cell, and yet more in malaria-infested cells, channels which probably are indigenous. However, single channel conductances, open probabilities and estimated number of channels/cell, give a conductance, far higher than the estimated whole cell conductance. A reconciliation of the results from cell suspensions with the observations on single cells or patches should be a high priority.

SESSION 1: MEMBRANE STRUCTURE AND STABILITY

Oral communication #1.1
Analysis of the relation between water content and osmotic fragility of human red blood cells

Anne Cueff[1], Agnieszka Dyrda[1], Guillaume Bouyer[1], Stéphane Egée[1], Rachel Seers[2], Alessandro Esposito[2], Jeremy Skepper[2], Teresa Tiffert[2], Virgilio L Lew[2], and Serge Thomas[1], [1]CNRS-UPMC, Station Biologique, Roscoff, France, [2]Department of Physiology, Development and Neuroscience, University of Cambridge, UK

Objectives: To test the role of Ca^{2+}-activated non-selective cation channels in red cell volume recovery after Gardos channel activation and associated cell shrinkage.

Design and methods: Osmotic fragility curves of human red blood cells exposed to relatively high and sustained Ca^{2+} loads are obtained by rapidly diluting cell samples into relatively large volumes of media with progressively reduced osmolalities.

Results: We present here an in-depth study of the water and ion content changes associated with dynamic shifts in osmotic fragility curves under a variety of experimental conditions. The results reveal that intracellular Ca^{2+} elevations have unique effects on the osmotic fragility of RBCs whose significance conflicts with the conventional interpretation of osmotic fragility data.

Oral communication #1.2
Stability and function of membrane nanotubes

Ales Iglic[1], Marusa Lokar[1], Henry Hägerstrand[2], Peter Veranic[3], and Veronika Kralj-Iglic[4], [1]Laboratory of Physics, Faculty of Electrical Engineering, University of Ljubljana, Ljubljana, Slovenia, [2]Department of Biology, Åbo Akademi University, Biocity, Åbo/Turku, Finland, [3]Institute of Cell Biology, Faculty of Medicine, University of Ljubljana, Lipičeva 2, SI-1000 Ljubljana, Slovenia, [4]Laboratory of Clinical Biophysics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Objectives: Communication between cells is crucial for proper functioning of multi-cellular organisms. The recently discovered membranous tubes, named tunnelling nanotubes, that directly bridge neighbouring cells, may offer a very specific and effective way of intercellular communication. The aim of the present work was to elucidate the possible physical mechanism of formation and mechanical stability of tubular membrane protrusions and nanotubes that bridge two neighbouring cells.

Design and methods: The formation and stability of nanotubes were studied experimentally (phase contrast, fluorescence and electron microscopy) and theoretically (modeling and simulations) in erythrocyte systems and in RT4 and T24 urothelial cell lines.

Results: We indicated that formation of nanotubes of erythrocyte membrane is driven by curvature-mediated self-assembly of anisotropic membrane nanodomains. The proposed mechanism of growth and stability of membrane tubular structures in erythrocytes is tested also in RT4 and T24 urothelial cell lines. Among others we have shown that tunelling nanotubes remain stable even after disintegration of the actin filaments within nanotubes by cytochalasin D. This indicates that nanotubular membrane protrusions and bridging nanotubes

0009-9120/$ - see front matter
doi:10.1016/j.clinbiochem.2009.09.001

may be mechanically stabilized by clustering of anisotropic (flexible) membrane nanodomains.

Oral communication #1.3
Regulation of erythrocyte survival by endothelin 1
Michael Föller[1], Manuel Braun[1], Syed Qadri[1], Berthold Hocher[2], and Florian Lang[1], [1]Department of Physiology, University of Tübingen, Germany, [2]Department of Pharmacology, Center for Cardiovascular Res., Charité, Berlin, Germany

Objectives: Endothelins are potent endothelium-derived mediators regulating vascular function. Erythrocytes are known to express the endothelin B (ETB) receptor . The present study explored the participation of the ETB receptor in the regulation of suicidal erythrocyte death.

Design and methods: Rescue mice lacking ETB receptor with normal neuronal expression ($ETB^{-/-}$) were compared to their wild type littermates. Furthermore, the role of the ETB receptor was tested by pharmacological manipulation. Phosphatidylserine exposure was detected by annexin V-binding.

Results: Energy depletion stimulated suicidal death of human erythrocytes, an effect significantly blunted by ETB receptor agonists endothelin 1 (500 nM) or sarafotoxin (10 nM). $ETB^{-/-}$ mice had significantly less erythrocytes than $ETB^{+/+}$ mice. $ETB^{-/-}$ erythrocytes were significantly more susceptible to stress-induced suicidal erythrocyte death than $ETB^{+/+}$ erythrocytes as determined from phosphatidylserine exposure. The observations disclose stimulation of the erythrocytic ETB receptor as a novel regulator of erythrocte survival *in vitro* and *in vivo*.

Oral communication #1.4
Analysis of the response of red blood cell membrane skeleton to different elementary shape transformations
Saša Svetina[1], Richard E. Waugh[2], and Boštjan Žekš[1], [1]Institute of Biophysics, Faculty of Medicine, University of Ljubljana, and Jožef Stefan Institute, Ljubljana, Slovenia, [2]Department of Biomedical Engineering, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA

Objectives: On the basis of known elastic behavior of spectrin and other red blood cell (RBC) skeleton constituents we intend to develop a consistent set of macroscopic constitutive relationships with which it will be possible to interpret, on a common basis, the results obtained on RBC elastic properties of normal and abnormal cells by different experimental setups.

Design and methods: We investigate the dependence of the skeleton redistribution on the type and extent of changes of its geometry and determine the corresponding energy changes. Elementary geometry transformations studied are between and within flat shapes, cylindrical shapes and sections of the sphere.

Results: The energy changes at described characteristic alterations of the skeleton geometry are presented for the constitutive relationships for the RBC skeleton derived for different previously proposed molecular models. The analysis reveals to what extent the effects of the RBC skeleton depend on the imposed geometrical constraints and how much on its elasticity.

Oral communication #1.5
Ca^{2+} content and phosphatidylserine exposure of red blood cells
Duc Bach Nguyen, Daniel Moersdorf, Lisa Wagner, Ingolf Bernhardt, Laboratory of Biophysics, Faculty of Natural and Technical Sciences III, Saarland University, Saarbruecken, Germany

Objectives: Investigation of phosphatidylserine (PS) exposure of erythrocytes caused by an increase of the intracellular Ca^{2+} content, which is induced by lysophosphatidic acid (LPA).

Design and methods: FACS measurements and single cell fluorescence microscopy, annexin-V FITC binding assay for PS determination, fluo-4 for Ca^{2+} content measurement.

Results: Treatment of human erythrocytes with 2.5–10 μM LPA leads to a significant PS exposure (up to 99% of cells) and cell shrinkage. This is due to (i) the activation of the Ca^{2+}-activated K^+ channel and (ii) the activation of the scramblase. PS exposure can be also stimulated by the addition of prostaglandin E_2, osmotic shock, oxidative stress and time of cell storage. Glucose depletion and replacement of chloride by methylsulphate have little effect. In contrast, sheep erythrocytes do not show PS exposure under the same conditions (except at high oxidative stress), probably due to the absence of the scramblase.

Oral communication #1.6
Evaluation of band 3 diffusion and spectrin compartment size by single particle tracking
Philip S. Low[1], Jeff Spector[2], Caitlin Sullivan[3], Rich Labotka[4], Ken Ritchie[2], and Gayani C. Kodippili[1], [1]Department of Chemistry, [2]Department of Physics, Purdue University, West Lafayette, IN, USA, [3] Department of Physics, University of Illinois, Urbana-Champaign, IL, USA, [4]University of Illinois Medical Center, Pediatric Hematology and Oncology, Chicago, IL, USA

Objectives: To define the nature of interactions between band 3 and the membrane cytoskeleton in normal and pathologic erythrocytes by monitoring the diffusion of single band 3 molecules in intact erythrocytes.

Design and methods: Quantum dots were conjugated specifically to band 3 via a DIDS-linker and their diffusion was monitored via high-speed video microscopy in intact healthy and pathologic erythrocytes (e.g. hereditary spherocytosis, hereditary elliptocytosis, hereditary pyropoikilocytosis, southeast Asian ovalocytosis, sickle cells, and hereditary hydrocytosis).

Results: Based on analysis of band 3 diffusion coefficients and compartment size data, at least two populations of band 3 were distinguishable in normal cells. Moreover, the band 3 diffusion profile was distinctly different between normal and pathologic erythrocytes, and also among all pathologic cells. The data demonstrate that single particle trafficking can be used to diagnose and characterize the structural defects in erythrocytes membranes with various hemolytic anemias.

Oral communication #1.7

Disturbed phospholipid scrambling in spur cell anemia

Petronella Maria Hilarius[1], Rob van Zwieten[1], Mark Rijke, Ferdi Bergkamp[2], Dirk de Korte[1], and Arthur Johannes Verhoeven[1], [1]Blood Cell Research, Sanquin Research, Amsterdam, [2]Spaarne Hospital, Hoofddorp, The Netherlands

Objectives: Spur cell anemia (SPA) is an acquired red cell defect associated with elevated free cholesterol levels in plasma and as a consequence high ratio of cholesterol to phospholipid (PL) in the erythrocyte membrane. We investigated the effect of elevated cholesterol on the ATP-dependent flippase activity, maintaining PL asymmetry, and the ATP-independent scrambling activity responsible for PS exposure in the RBC membrane.

Design and methods: RBC from a SPA patient and a control were analyzed for morphology, osmotic fragility, ATP levels, cholesterol and PL content, flippase activity (by NBD-PS translocation) and Ca^{2+}-induced scrambling (by PS exposure and NBD-PC translocation). In artificial cholesterol-loaded and depleted RBC the same analyses were performed.

Results: An elevated chol/PL ratio, as observed in spur cells, profoundly inhibits scrambling activity in the RBC membrane. Apparently, high cholesterol not only induces RBC deformation, but it also inhibits the exposure of PS, an important death signal.

Oral communication #1.8

Erythrocyte removal signals in blood bank and clinic

Malou Hermkens[1], Judith Cluitmans[1], Sam van Beuningen[1], Mark Stappers[1], Sip Dinkla[2], Vera Novotný[2], and Giel Bosman[1], [1]Department of Biochemistry, [2]Department of Blood Transfusion and Transplantation Immunology, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Sanquin Blood Supply Foundation, Nijmegen, The Netherlands

Objectives: To characterize the removal signals and the relevant pathways, we examined exposure of phosphatidylserine (PS) and changes in band 3 on erythrocytes and their vesicles during storage, and in patients with various erythrocyte membranopathies.

Design and methods: Erythrocytes were stored in blood bank conditions, and separated according to density. Vesicles were isolated by differential centrifugation. Exposure of PS was measured using fluorescent annexin V. Changes in band 3 structure and autoantigen activity were investigated using binding and anti-band 3 antibodies and autologous IgG, respectively, and flow cytometry.

Results: PS-exposure and amount of cell-bound IgG increase with storage, and in patients with membranopathies, mostly in the densest erythrocytes. Storage vesicles expose PS, and most vesicles contain IgG. Treatment with band 3-reactive reagents indicates that storage is associated with changes in band 3 structure and autoantigen reactivity. Band 3 is a sensitive, informative parameter of erythrocyte quality and clinical condition.

Oral communication #1.9

Changes in membrane asymmetry upon cryopreservation of red blood cells with different cryoprotectants

Olga A. Kofanova[1,2], Nina G. Zemlyanskikh[2], Heiko Zimmermann[3], and Ingolf Bernhardt[1], [1] Laboratory of Biophysics, Saarland University, Saarbruecken, Germany, [2]Institute for Problems of Cryobiology and Cryomedicine, Kharkov, Ukraine, [3]Cryobiophysics and Cryotechnology, Fraunhofer IBMT, St. Ingbert, Germany

Objectives: Red blood cell (RBC) stability during cryopreservation is depending on the modification of structure membrane by cryoprotectant agents (CPAs) and low temperature.

Design and methods: The aim of this work was the investigation of asymmetrical distribution changes of phosphatidylserine (PS) during RBC cryopreservation with CPAs glycerol and polyethylene glycol (PEG-1500) by flow cytometry.

Results: The PS exposure of RBCs has been investigated after incubation with CPAs before freezing. Glycerol didn't affect significantly the PS distribution up to 24 h of incubation. The number of annexin-V labelled cells reached 10% and 40% after 1 h and 24 h of PEG incubation, respectively. After freeze–thawing of RBC suspensions the number of cells which bind annexin-V was 15% and 32% in the presence of glycerol and PEG, respectively. Following RBC incubation into Ringer-glucose medium, the difference of PS exposure in the membrane of cells cryopreserved in the presence of glycerol and PEG has been detected.

Oral communication #1.10

The modifications of the membrane structure of red blood cells under the action of pulsed electrical fields and gamma-radiation

Elena Kozlova[1], Alexander Chernysh[1], and Victor Moroz[2], [1]Moscow Medical Academy, [2]Research Institute of General Reanimatology, Moscow, Russsia

Objectives: The objective is the study of the defects in erythrocyte membranes, arising under the action of gamma-radiation and pulsed electrical fields.

Design and methods: Human red blood cells were subjected to a pulsed electrical field of 1500 V/cm and/or gamma radiation from super low doses (10 cGy) to high doses (600 Gy). The methods of calibrated electroporation and Atomic Force Microscopy were used. Cells fixed with glutaraldehyde and whole blood were studied.

Results: Electrical field and gamma-radiation induced the additional defects in erythrocyte membrane and changed the parameters of the surface roughness. The height of roughness was 0.2–5 nm, the period was 30–300 nm.

Oral communication #1.11

Band 3 phosphorylation promotes its clustering and erythrocyte membrane destabilization

Antonella Pantaleo, Emanuela Ferru, Rosa Vono, Franca Mannu, Giuliana Giribaldi, and Franco Turrini, University of Torino, Torino, Italy

Objectives: Characterize the mechanism underlying band modifications leading to its phosphorylation and clustering.

Design and methods: Band 3 phosphorylative modifications following oxidative treatments have been studied using western blots with anti-phosphotyrosine antibodies. To co-associate protein bands the reading was performed by dual source IR fluorescence scanner: Band 3 glycosilation and mapping of its phosphorylation sites have been studied by mass spectrometry (MALDI-TOF, LC-MS/MS). Isolation of band 3 containing high molecular weight aggregates was performed by size exclusion HPLC. Syk and Lyn membrane translocations were studied using specific monoclonal antibodies.

Results: Following hemichrome binding, we demonstrated that oxidative band 3 modifications caused selective Syk kinase binding to disulfide cross-linked band 3 leading to its phosphorylation. A fraction of under-glycosilated band was preferentially cross-linked and formed high molecular weight complexes binding naturally occurring IgG. Band 3 phosphorylation apparently causes loss of cytoskeletal cohesion. The decrease of its lateral constraint facilitate large band 3/ hemichrome cluster.

SESSION 2: ERYTHROPOIESIS, IRON AND PORPHYRIAS

Oral communication #2.1

Effects of manipulating tumor oxygenation and iron metabolism by erythropoietin on tumor growth in vivo

Catherine Dujardin, Olivier Dengis, Raafat Stephan, and Yves Beguin, Hematology Unit, GIGA-Research, University of Liège, CHU Sart-Tilman, Liège, Belgium

Objectives: Recombinant human erythropoietin (rHuEpo) can correct anemia and improve quality of life in cancer patients, but its effect on survival remains controversial.

Design and methods: We examine the effects of manipulating tumor oxygenation and iron metabolism by treatment with rHuEpo on myeloma tumor growth.

Results: We first used the multiple myeloma MOPC-315 model in BALB/c mice. Animals challenged with a SC tumor received either saline or were treated with rHuEpo, oral iron or iron plus rHuEpo. No significant effect on tumor growth rate and survival was observed. Mice challenged with cells administered intravenous were divided in the same four groups, and again no significant effect on survival was observed. These results demonstrate that rHuEpo and iron did not modulate *in vivo* tumor growth rate and survival of MOPC-315 tumor-bearing mice. These experimental data support the safety profile of rHuEpo and iron in the treatment of myeloma patients.

Oral communication #2.2

Alterations of systemic and muscle iron metabolism in human subjects treated with low dose recombinant erythropoietin

Paul Robach[1], Stefania Recalcati[2], Domenico Girelli[3], Cecilia Gelfi[4], Niels J. Aachmann-Andersen[5], Jonas J. Thomsen[5], Anne M. Norgaard[5], Alessandra Alberghini[2], Natascia Campostrini[3], Annalisa Castagna[3], Agnese Viganò[4], Paolo Santambrogio[6], Tibor Kempf[7], Kai C. Wollert[7], Stéphane Moutereau[8], Carsten Lundby[5,9], and Gaetano Cairo[2], [1]Ecole Nationale Ski et Alpinisme, Chamonix, France, [2]Department Human Morphol. and Biomed. Sci., Univ. Milan, Italy, [3]Dept. of Clin. and Exp. Medicine, Univ. Verona, Italy, [4]Department Sci. and Biomed. Technol., Univ. Milan, Italy, [5]The Copenhagen Muscle Res. Centre, Univ. Copenhagen, Denmark, [6]Dibit, H.S. Raffaele, Milano, Italy, [7]Department of Cardiol. and Angiol., Hannover Med. School, Germany, [8]Lab. Biochim., Hôp. Henri-Mondor, Créteil, France, [9]Department Sport Sci., Univ. Århus, Denmark

Objectives: The high iron demand associated with enhanced erythropoiesis during high-altitude hypoxia leads to skeletal muscle iron mobilisation (Robach et al. *Blood* 2007). Here we investigated the effect of enhanced erythropoiesis on systemic and muscle iron metabolism under non-hypoxic conditions.

Design and methods: Blood, urine (for hepcidin determination) and muscle biopsies (for evaluation of mRNAs and proteins of iron metabolism) were taken at various time points in healthy volunteers treated with recombinant erythropoietin (rhEpo) for one month.

Results: rhEpo treatment, which stimulated erythropoiesis and bone marrow iron use, was also associated with a prompt and considerable decrease in hepcidin. The increased iron use and reduced hepcidin suggested increased iron mobilisation, but we found increased muscle iron and ferritin levels. The muscle expression of transferrin receptor and ferroportin was up-regulated. In conclusion, under rhEpo stimulation, skeletal muscle accumulates iron despite the remarkable hepcidin suppression, which may be directly mediated by rhEpo.

Oral communication #2.3

In vivo and *in vitro* effect of ribavirin and interferon on erythropoiesis in patients with hepatitis c

Luisa Ronzoni[1], Alessandra Colancecco[1], Alessandra Cattaneo[2], Alessio Aghemo[3], Sara Monico[3], Maria Grazia Rumi[3], Massimo Colombo[3], Maria Domenica Cappellini[1], [1]Department of Internal Medicine, [2]Cytometry Laboratory, Department of Regenerative Medicine, [3]First Division of Gastroenterology; Fondazione Ospedale Maggiore Policlinico IRCCS, University of Milano, Milano, Italy

Objectives: The combination of Peg-Interferon (Peg-IFN) plus Ribavirin (RBV) is the standard of care in patients with hepatitis C. RBV probably cause hemolytic anemia. We studied the mechanisms underlying anemia.

Design and methods: We studied 18 HCV patients analyzing along treatment hematological parameters and the number of their pheripheral BFUe; in vitro, we evaluated the effects of RBV and Peg-IFN on erythroid differentiation of CD34 pheripheral blood cells.

Results: All patients showed a significantly reduction in hemoglobin levels, but only 3 of them developed hemolytic anemia. BFUe number decreased in patients without signs of hemolysis while increased in patients with hemolytic anemia. Erythroid differentiation in vitro of CD34 cells was significantly

reduced in the presence of Peg-IFN and even more with the combination therapy. In conclusion, Peg-IFN and RBV both have an inhibitory effect on erythroid differentiation. The hemolysis so far attributed to RBV is not the major mechanism underlying anemia.

Oral communication #2.4

Iron, zinc and aluminium content of serum ferritin in hyperferritinemic patients and in healthy subjects

Bianca Maria Ricerca[1], Cristina Rossi[2], Pier Luigi Spada[3], Alessandro Alimonti[5], Beatrice Bocca[5], Marzia Marino[1], Maria Grazia Bocci[4], and Pasquale De Sole[2], [1]Department of Hematology, [2]Department of Clinical Biochemistry, [3]Department of Surgery, [4]Department of Anaesthesiology and Intensive Care, Catholic University of Sacred Heart, Rome, Italy, [5]Department of Environment and Primary Prevention, National Health Institute, Rome, Italy

Objectives: Iron Content of Ferritin (ICF) differs in hyperferritinemic patients according to the clinical setting. Since s-F can bind also other metals, we verified their influence on ICF.

Design and methods: 16 Hereditary Hemochromatosis (HH) patients, 14 septic (SE), 13 hemodialysed (HD) and 10 plasma pools from 100 healthy subjects were examined. Iron, Al, and Zn of sF were measured by mass spectrometry (atoms/ferritin molecule).

Results: If Iron, Al and Zn are cumulated, there isn't any significant difference in the three hyperferritinemic groups (2377±1182 HH; 2873±1252 SE; 1890±888 HD, p=NS). While zinc parallels iron in all groups, Al is significantly higher in HD and healthy subjects. The ratio among Al, Iron, Zn is almost 1:1:1 in SE and HH while in HD and healthy subjects is approximately 6:1:1.

Oral communication #2.5

Molecular mechanisms of genetic iron deficiency iron refractory anemia (IRIDA)

Laura Silvestri, Alessia Pagani, Antonella Nai, Flavia Guillem, Gina De Falco, Achille Iolascon, Carole Beaument, Bernard Grandchamp, and Clara Camaschella, Vita-Salute San Raffaele University, Istituto Scientifico San Raffaele, Milan, Italy

Objectives: Hepcidin is the master regulator of iron homeostasis. It increases in iron-overload and inflammation and decreases in iron-deficiency, hypoxia and erythropoietic-expansion. *TMPRSS6* is a negative regulator of hepcidin *in vivo* and its mutations are associated with IRIDA. We have recently shown that membrane-hemojuvelin (HJV), which activates hepcidin as a BMP (Bone Morphogenetic Protein) coreceptor, is the substrate of matriptase-2. Here we extend our studies on new *TMPRSS6* mutations associated with IRIDA.

Design and methods: Wild-type and *TMPRSS6*-causative-mutants were analyzed in the presence of HJV. The mutant ability to inhibit hepcidin was assessed in cells transfected with a hepcidin promoter-luciferase-reporter-construct.

Results: Matriptase-2 variants interact with HJV, have partial inhibitory activity on HJV-mediated hepcidin activity and retain only partial ability to cleave membrane-HJV, except LDLRA mutants that are fully inactive. We confirm that HJV is a substrate of matriptase-2. The inability to cleave membrane-HJV might explain the clinical features of IRIDA.

Oral communication #2.6

GATA-2 and GATA-6 involvement in hydroxy-carbamide action on endothelial cells in sickle cell disease

Pauline Lansiaux, Sandrine Laurance, Michèle Hauchecorne, Emmanuelle Verger, Claudine Lapouméroulie, and Jacques Elion, Inserm U763, Hôpital Robert Debré, Paris, France

Objectives: To decipher pathway(s) by which hydroxycarbamide (HC) decreases adhesion properties of endothelial cells (ECs) in sickle cell disease, and specifically to validate two candidate genes, namely transcription factors GATA-2 and GATA-6, identified by microarray analysis as potential intermediates in the HC-induced decreased expression of endothelial adhesion proteins (EAP).

Design and methods: GATA-2 and 6 expression was measured at the mRNA level by RQ-PCR and at the protein level by western blot in HC-treated ECs from the micro-(TrHBMEC) and macro-(HUVEC) circulations for 5–48 h in basal and inflammatory conditions.

Results: In TrHBMEC, GATA-2 and -6 are time-dependently decreased by HC in basal and inflammatory conditions (>50% at 48 h, $p<0.001$). In HUVEC, results differ depending upon the experimental conditions. This results are in agreement with the observed HC effect on GATA-2/6-dependent EAP genes. Functional HC effect on GATA-2/6 binding to EAP GATA-responsive elements is currently tested by gel retardation assays.

Oral communication #2.7

C-terminal deletion in the ALAS2 gene causes X-linked erythropoietic protoporphyria (EPP) despite of wild type fech gene

Valentina Brancaleoni, Elena Di Pierro, Dario Tavazzi, and Maria Domenica Cappellini, Department of Internal Medicine Fondazione Ospedale Maggiore Policlinico IRCCS, University of Milan, Milan, Italy

Objectives: Considering that mutational analysis fails to detect FECH mutations in about 20% of EPP families, in this study we re-examined 7 Italian unrelated *FECH*-negative EPP families.

Design and methods: The ALAS 2 exon 11 has been amplified by PCR and subjected to direct automated sequencing.

Results: Among 4 families, 6 males and 3 females carried a deletion c.1706–1709 delAGTG. Out of three unrelated females, one was asymptomatic while the other two showed severe photosensitivity. This remarkable heterogeneity of phenotypes between females could result from X-chromosome

inactivation. Otherwise we found no evidence that X-inactivation could lead to a milder disease in symptomatic females. In fact, these latter showed a similar erythrocyte protoporphyrin concentration and liver involvement as symptomatic males. The molecular defect in 3 *FECH*-negative EPP families remains still unknown, suggesting that new genes could be involved in causing Porphyria phenotypes.

Oral communication #2.8

Functional properties of recombinant α-Hb Questembert (H14 Pro) and α-Hb Caen (H15gly), obtained by coexpression with the alpha hemoglobin stabilizing protein (AHSP)

Vèronique Baudin-Creuza[1], Corinne Vasseur[1], Elisa Domingues[1], Thomas Brillet[1], Henri Wajcman[2], and Michael C. Marden[1], [1]INSERM Unité 779, University of Paris XI, Le Kremlin-Bicêtre, France, [2]INSERM Unité 955, Hôpital Henri Mondor, Créteil, France

Objectives: Hb Questembert α131Ser→Pro and Hb Caen α132Val→Gly are unstable variants with a thalassemic like syndrome. Their structural abnormality is located in the H helix, a region which interacts with the AHSP and β-Hb. We report the studies of these α-Hb variants with partner proteins.

Design and methods: These mutants were co-expressed with AHSP as fusion proteins with glutathione-S-transferase (GST). The GST-AHSP/GST-α-Hb complex was purified by affinity chromatography and analysed by SDS-PAGE. The function was studied by kinetics of CO rebinding after photodissociation.

Results: The mutated α-Hbs are coexpressed with AHSP with normal yields. After solubilisation, less of mutated GST-α-Hbs are recovered. These GST-AHSP/GST-α-Hb complexes have an abnormal function compared to the normal complex. Before and after addition of β-Hb, the mutated complexes with AHSP and mutant Hb tetramers display essentially the rapid R like kinetics of CO rebinding. These results suggest a modified interaction with AHSP and/or an impaired binding of β-Hb.

Oral communication #2.9

Role of adhesion molecule ICAM-4 in erythropoiesis

Jonathan Villalobos, Gloria Lee, Sarah Short, and Joel Anne Chasis, Life Sciences, University of California, Lawrence Berkeley National Laboratory, Berkeley, CA, USA

Objectives: Erythropoiesis occurs within erythroblastic islands, comprised of erythroblasts surrounding a macrophage. We are exploring the function of erythroblast adhesion molecule ICAM-4. Bone marrow islands are markedly decreased in ICAM-4 knockout mice. The current studies asked whether the decreased islands impair response to stress erythropoiesis.

Design and methods: WT and ICAM-4 null mice were administered Epo on days 1-3, 10 and 11. Reticulocytes were counted on new methylene blue smears. Freshly harvested islands were quantified by immunofluorescent microscopy employing erythroid-specific TER119, macrophage-specific F4/80 and a DNA probe.

Results: WT and KO reticulocyte counts increased 3-fold by day 7 and nadired on day 10. Island number was significantly less in KO compared to WT at the nadir of the reticulocyte response. Strikingly, after the second set of Epo, the KO reticulocyte response was much lower than WT. We conclude that ICAM-4 is required for an appropriate response to prolonged stress erythropoiesis.

SESSION 3: NOVEL TECHNOLOGIES

Oral communication #3.1

Red blood cell structure and dynamics explored with digital holographic microscopy

Pierre Marquet[1,2], Daniel Boss[2], Benjamin Rappaz[2], Pascal Jourdain[2], Christian Depeursinge[3], and Pierre Magistretti[1,2], [1]Centre de Neurosciences Psychiatriques, University of Lausanne, Prilly-Lausanne, Switzerland, [2]Brain Mind Institute, [3]Imaging and Applied Optics Institute, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

Objectives: Red blood cell membranes present a spontaneous flicker phenomenon at a nanometric scale. Although discovered a long time ago, the origin of this flickering is not yet fully understood and its characteristics remain difficult to investigate.

Design and methods: We have developed a digital holographic microscopy (DHM) technique that allows obtaining, from a single recorded hologram, quantitative phase images of living cell with a nanometric axial sensitivity. Specifically, the optical phase shift induced by the specimen on the transmitted wave front can be regarded as a powerful endogenous contrast agent, depending on both the thickness and the refractive index of the sample, which can be measured separately.

Results: The DHM nanometric axial and microsecond temporal sensitivities have allowed to measure and dynamically characterize the red blood cell membrane fluctuations (CMF) on the whole cell surface. In addition, CMF has been successfully decomposed into a series of spatially well defined modes.

Oral communication #3.2

Investigation of erythrocytes cell-cell adhesion using holographic optical tweezers

Patrick Steffen, and Christian Wagner, Department of Physics, Faculty of Natural and Technical Sciences II, Saarland University, Saarbrücken, Germany

Objectives: In the classical model, the role of red blood cells (RBCs) in blood clot formation is thought to be passive. It is supposed that they get caught into a fibrin-network, generated in the clotting process, just for reasons of geometrical restrictions. Additionally, it is commonly believed that there exist no adhesion forces among the cells. The main part in clot formation take activated platelets. Prostaglandin E_2 (PGE_2) and lysophosphatidic acid (LPA) are messengers released from these activated platelets. Treating RBCs with LPA or PGE_2 leads to a Ca^+ influx into the cells. The consecutive rise of internal

calcium level activates the scramblase protein whereby the negatively charged phosphatidylserine (PS) gets to the outer leaflet of the cell membrane (these results are given in a separate presentation by D.B. Nguyen et al.). Thus the objective is to investigate the contribution of RBCs in blood clot formation.

Design and methods: In order to test the hypothesis we have built up an integrated microfluidic holographic optical tweezers setup to study this cell adhesion.

Results: Measurements with the calcium Ionophor A23187 and LPA showed that RBCs tend to adhere to each other when intracellular calcium levels are increased. Thus, we postulate that the response of RBCs on PGE_2 and LPA reveals a direct and active participation of these cells in blood clot formation.

Oral communication #3.3

Rheological profile and direct imaging of erythrocytes affected by genetic disorders

Patrizia Caprari[1], Anna Tarzia[1], Giorgio Mojoli[2], Desirè Di Silvio[1], and Maria Cristina Martorana[3], [1]Dipartimento di Ematologia, Oncologia e Medicina molecolare, Istituto Superiore di Sanità, Rome, Italy, [2]Miane, Treviso, Italy, [3]Centro Aziendale Produzione Emocomponenti, A.O. S. Camillo-Forlanini, Rome, Italy

Objectives: The contribution of red blood cells (RBCs) to flow behaviour of blood is closely related to cell deformability and aggregation. In this work the behaviour of RBCs affected by thalassemia, hereditary spherocytosis and hereditary elliptocytosis is analysed by the Rheo-Microscope system that simultaneously allows erythrocyte rheological characterization and direct imaging.

Design and methods: The study was carried out by the Rheo-Microscope (Anton Paar, Germany) constituted by the rheometer Physica MCR301, placed on an optical microscope and a CCD camera. Blood samples have been tested at shear rates between 1 and 250 s^{-1} according to ICSH. Direct imaging of RBCs under shear flows was captured by imaging software.

Results: Altered rheological profiles have been found in diseased RBCs. The images obtained by microscope showed the formation of aggregates, at low shear rates, and the progressive complete disaggregation under flow conditions. Heterogeneous flow behaviour with different aggregation–disaggregation patterns has been observed in pathological RBCs.

Oral communication #3.4

Advanced technologies for noninvasive prenatal diagnosis of beta-thalassemia

Silvia Galbiati[1], Francesca Bruno[1], Vincenza Causarano[1], Gabriella Restagno[2], Marcella Chiari[3], Francesco Damin[3], Cristina Curcio[4], Manuela Seia[4], Maurizio Ferrari[1,5,6], Laura Cremonesi[1], [1]Genomic Unit for the Diagnosis of Human Pathologies, Center for Genomics, Bioinformatics, and Biostatistics, San Raffaele Scientific Institute, Milano, Italy, [2]Department of Patologia Clinica, A.O.O.I.R.M.-S. Anna, Torino, Italy, [3]Istituto di Chimica del Riconoscimento Molecolare (ICRM) – C.N.R, Milano, Italy, [4]Ospedale Maggiore Policlinico Mangiagalli Regina Elena, Milano, Italy, [5]Università Vita-Salute San Raffaele, Milano, Italy, [6]Diagnostica e Ricerca San Raffaele SpA, Milano, Italy

Objectives: Fetal DNA in maternal plasma represents a source of fetal genetic material obtained noninvasively. Our goal is to develop advanced methodologies for noninvasive prenatal diagnosis of genetic diseases.

Design and methods: We first evaluated different systems (microchip, pyrosequencing and direct sequencing) coupled with the use of peptide nucleic acids (PNAs) to inhibit the amplification of maternal DNA and enrich the mutant fetal DNA of paternal origin. To implement our strategy, we developed a highly sensitive microarray based on a new slide coated with an innovative functional polymer.

Results: With the first approach we performed 41 prenatal diagnoses of beta-thalassemia on maternal plasma, with total concordance of results obtained on fetal DNA extracted from chorionic villi. By the use of the new substrate, the sensitivity in detecting the fetal mutated allele was higher allowing the identification of fetal alleles also in the absence of PNAs.

Oral communication #3.5

A new drug form of blood coagulation factor IX: red blood cell-entrapped factor IX

Elena Ivanovna Sinauridze, Tatyana Alekseevna Buimo, Elena Viktorovna Kulikova, Irog Igorevich Shmyrev, and Fazoil Inoyatovich Ataullakhanov, National Reseach Center for Hematology, Russian Academy of Medical Sciences, Moscow, Russia

Objectives: Design of new erythrocyte-included coagulation factor IX (FIX) as a pharmacological form for prolonging its lifetime in circulation and decreasing the risk of immune response.

Design and methods: Factor IX was biotinylated and included in erythrocytes (RBC) by original stepwise dialysis method. The pharmacokinetics of free biotinylated FIX (FIXbiot) and FIXbiot entrapped in RBCs were compared in volunteers, who gave the informed consent. The concentrations of FIXbiot in plasma and RBC lysates before and up to 15 days after injection were measured using a sandwich ELISA.

Results: The yield of loaded cells averaged $55 \pm 10\%$. The FIXbiot concentration in lysates of the loaded cells was, on average, $24.3 \pm 10.5\%$ of its initial concentration in the suspension. The lifetime of the RBC-based form of FIX in the circulation was 5–10 times longer than that of the free form.

SESSION 4: MEMBRANE TRANSPORT AND METABOLISM

Oral communication #4.1

Cation conductance through the anion exchanger AE1. on the way to draw the cation pore

Franck Borgese, Stephen Walsh, Nicole Gabillat, Bernard Pellissier and Hélène Guizouarn, Laboratoire de Biologie et

Physiopathologie des Systèmes Intégrés, Université de Nice, Nice, France

Objectives: Recently, several point mutations of AE1 (Cl/HCO_3 exchanger of red cell) have been identified that modify the transport function of the anion exchanger. Some convert the exchanger to a non selective cation channel (mutations related to stomatocytosis), other add a cation conductance to the anion exchange function (mutations related to dRTA). All mutations are localised around transmembrane domains 8 and 9 of the protein. Experiments were carried on to localise the cation pore in the AE1 spanning domain.

Design and methods: Electrophysiological parameters of the cation conductance were measured using the 2-electrode voltage clamp technique in Xenopus oocytes expressing mutated AE1. Cation influx was measured using an absorbance atomic spectrometry apparatus.

Results: The 3D organisation of the transmembrane domains that contain mutated amino acids in such a way to form a funnel gave us the ability to point out new residues involved in the cation conductance.

Oral communication #4.2

Gardos and anionic channels activation induced by local membrane deformation in intact human red blood cells

Agnieszka Dyrda[1], Urszula Cytlak[1,2], Anna Ciuraszkiewicz[1,2], Anne Cueff[1], Agnieska Lipinska[1], Guillaume Bouyer[1], Stéphane Egée[1], Poul Bennekou[3], and Serge Thomas[1], [1]CNRS-UPMC, Station Biologique, Roscoff, France, [2]Institute of Physics, Wroclaw University of Technology, Poland, [3]Institute of Molecular Biology and Physiology, University of Copenhagen, Denmark

Objectives: To test to what extent membrane deformation of human red blood cell could induce channel activity.

Design and methods: We used the cell-attached configuration of the patch-clamp technique of electrophysiology. Deformation was obtained by depression of 10 mm Hg applied for less than 10 s in glass micropipettes brought in contact.

Results: We present electrophysiological evidence that Ca^{2+}-sensitive K^+ channel are transiently activated when seal formation induces membrane deformation and that this phenomenon can result only from activation of a permeability pathway with a finite Ca^{2+}conductance (PCa). This transient activity generates secondary transient anionic channel activity.

Oral communication #4.3

Water permeability of red blood cells from various vertebrate species and its physiological significance

Gheorghe Benga, Dept. of Cell and Molecular Biology, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

Objectives: To get a better understanding of the physiological significance of water channel proteins, later called aquaporins (AQP) from red blood cells (RBCs).

Design and methods: The water diffusional permeability (P_d) and its activation energy ($E_{a,d}$) was measured in RBCs of over 30 vertebrate species.

Results: Based on P_d and $E_{a,d}$ the species investigated could be divided in several groups. The chicken and echidna RBCs had the lowest Pd and the highest $E_{a,d}$; this indicates that no functional AQPs are present. The human RBC had $P_d$values $\sim 4x10^{-3}$ cm/s at 25°C and $6x10^{-3}$ cm/s at 37°C and $E_{a,d} \sim 25$ kJ/mol. The RBCs from cow, sheep, horse and elephant had slightly lower $P_d$values, camel and alpaca RBCs slightly higher values. The RBCs from large macropodid marsupials had values of $P_d, \sim 6 \times 10^{-3}$ cm/s at 25°C and 8×10^{-3} cm/s at 37 °C. The RBCs from other marsupial species and from rat, mouse, guinea pig, rabbit RBCs had $P_d$values roughly twice higher. It appears that AQPs in the RBCs ensure the rate of exchange of water across the membrane required in various animals in relation to their physical activity and metabolic rate.

Oral communication #4.4

New insights into RBC metabolism during storage

Patrick Burger, Robin van Bruggen, Herbert Korsten, Arthur J. Verhoeven, Eva Rombout, and Dirk de Korte, Sanquin Blood Supply Foundation, Amsterdam, The Netherlands

Objectives: Several characteristics of red blood cells (RBC) change during *in vitro* storage at 4°C. Here we investigated the possibility to prevent metabolic changes induced by storage by application of a chloride-free storage medium.

Design and Methods: RBC units were prepared in either standard storage medium SAGM (saline, adenine, glucose, mannitol; pH 6.2) or experimental medium PAGGGM (phosphate, adenine, guanosine, glucose, gluconate, mannitol; pH 8.2), and metabolically characterized during 49 days of cold storage.

Results: RBC in PAGGGM had higher 2,3-DPG and ATP levels throughout storage despite having a similar intracellular pH. This contradicts the hypothesis in the literature that 2,3-DPG levels are primarily determined by the intracellular pH. Initial glucose-6-phosphate levels (G6P) were higher in RBC suspended in SAGM, but this difference was reversed upon storage. We conclude that PAGGGM stimulates at least two steps in glycolysis, one of which is hexokinase. These effects are not mediated by changes in the intracellular pH.

Oral communication #4.5

NMDA receptors in mammalian erythrocytes

Anna Bogdanova[1], Asya Makhro[1], Jeroen Goede[2], Jue Wang[3], Alexander Boldyrev[4], Max Gassmann[1] and Lars Kaestner[3], [1]Institute of Veterinary Physiology and Zurich Centre for the Integrative Human Physiology (ZHIP), University of Zurich, Zurich, Switzerland; [2]University Hospital Zurich, Clinic of Hematology, Zurich, Switzerland, [3]Anatomy and Cell Biology, Saarland University, University Hospital, Homburg, Germany, [4]International Biotechnology Centre, Department of Biochemistry, Moscow State University, Moscow, Russia

Objectives: This study was designed to characterize the presence and function of the NMDA receptors in mammalian erythrocytes.

Design and methods: Rat and human erythrocytes were used to monitor presence and function of the NMDA receptors using immunoblotting and ^{3}H-MK-801. The effects of agonists and antagonists of the receptors on ouabain-insensitive Cl-independent K^+ influx and Ca^{2+} influx were assessed using radioactive tracers and microfluorescent imaging.

Results: NMDA receptors are present in human and rat erythrocyte membranes. Their number varied depending on gender and cell age and was exceptionally high in erythrocytes of patients with sickle cell anemia. Activation of the receptors with NMDA or homocysteic acid caused rapid Ca^{2+}uptake and hemolysis. Both processes could be blocked by pretreatment of the cells with NMDA receptor antagonists MK-801 or memantine. We suggest that NMDA receptor may contribute to the cell clearance and hemolytic crisis in patients with sickle cell anemia.

SESSION 5: GENETIC DISORDERS OF THE RED CELL

Oral communication #5.1

Increased levels of pyruvate kinase (PK) antigen in red blood cells of patients with pk deficiency

Brigitte A. van Oirschot, Wouter W. van Solinge, and Richard van Wijk, Laboratory for Red Blood Cell Research, Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, Utrecht, The Netherlands

Objective: Pyruvate kinase (PK) deficiency is a rare red blood cell (RBC) enzymopathy causing hereditary non-spherocytic hemolytic anemia. The disease shows a marked variability in clinical expression. In order to better understand the genotype-phenotype correlation as well as the enzyme's structure and function, we developed a method to measure PK antigen levels.

Design and methods: In a group of PK-deficient patients the amount of RBC PK was quantified. Quantitative measurements of RBC PK was performed by ELISA using rabbit antibodies against the A and C domains of human RBC PK.

Results: Remarkably, in this group the loss of PK enzymatic activity was not accompanied by a quantitative reduction in the amount of PK. Indeed, in about half of the patients tested, PK amounts were increased. These results suggest that in some way deficiency of PK is able to induce an (compensatory) increase of PK synthesis, most likely during erythroid maturation.

Oral communication #5.2

Analysis of red cell membrane proteins by capillary electrophoresis

Andrea Mosca[1], Renata Paleari[1], Lorena Mosca[1], Anna Marcello[2], Cristina Vercellati[2], and Alberto Zanella[2], [1]Dip. Scienze e Tecnologie Biomediche, Università degli Studi di Milano; [2]U.O. Ematologia 2, IRCCS Fondazione Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Milano, Italy

Objectives: To develop a method based on SDS-capillary gel electrophoresis for the quantitative analysis of erythrocyte membrane proteins.

Design and methods: Red cell ghosts prepared from healthy subjects ($n=20$), as well from some patients affected by hereditary spherocitosis due to spectrin deficiency or CDA II were analyzed. The analyses were carried out with a Beckman Coulter ProteomeLab PA800 capillary electrophoresis, using an uncoated silica capillary (i.d. 50 μm × 20 cm).

Results: Seven major erythrocyte membrane proteins were separated and identified: α-and β-spectrin, bands 3, 4.1, 4.2, 5 and 6. Reproducibility (expressed as CV) of the migration times were between 0.1% and 0.3%. Reproducibility of protein quantification, expressed as ratios to band 5, were between 3.6% and 7.6%. The results on patients with membrane defects were in agreement with the results obtained with traditional SDS-PAGE. The method represents therefore a promising approach to the study of red cell membrane disorders.

Oral communication #5.3

Biochemical and molecular studies on undiagnosed cases of inherited hemolytic anaemia and methaemoglobinemia in India

Prashant Warang, Prabhakar Kedar, Anita Nadkarni, Sona Nair, Ghosh Kanjaksha, and Roshan Colah, National Institute of Immunohaematology, (Indian Council of Medical Research), K.E.M. Hospital campus, Parel, Mumbai, India

Objectives: To investigate for RBC membranopathies, enzymopathies and unstable hemoglobins in undiagnosed inherited hemolytic anaemias and methaemoglobinemias.

Design and methods: 158 cases were investigated: RBC membrane defects (79), enzymopathies/unstable hemoglobins (66), methaemoglobinemias (13). Membrane defects was assessed by flow-cytometry and SDS PAGE, enzymopathies by spectrophotometric assays and unstable haemoglobins by heat instability. RFLP, SSCP and DNA sequencing were used for molecular characterization.

Results: Membrane defects were present in 54 patients (two had Band 3 protein abnormalities). Nine cases showed pyruvate kinase deficiency [1436G → A mutation was common], one had glucose phosphate isomerase deficiency with neurological abnormalities and one showed basophilic stippling with reduced pyrimidine5′-nucleotidase activity. Unstable haemoglobins were observed in 2 cases. [Homozygous Hb Sallanches [α104 (Cys → Tyr)] and Hb Koln [β98 (Val → Met)]. NADH-b5R deficiency was found in 7 cases of methemoglobinaemia [Three novel (Arg49Trp, Gly154Glu, Ile117Thr) and three other mutations]. Pyruvate kinase deficiency, NADH-b5R deficiency and unstable hemoglobins are not uncommon in India.

Oral communication #5.4

ENERCA 3: European reference network of expert centers in rare anaemias

Joan-Lluis Vives Corrons, and Maria del Mar Mañú Pereira (On behalf of ENERCA consortium), Red Cell Pathology Unit, Hospital Clínic i Provincial, University of Barcelona, Barcelona, Spain*

Objectives: ENERCA 3 is the third phase of two previous Projects (ENERCA 1 and 2) starting in 2002 co-funded by the European Commission for the establishment of an "European Network for Rare and Congenital Anaemias" (www.enerca.org), to create an European Reference Network (ERN) of Expert Centres (EC) in Rare Anaemias (RA) as a platform for health professionals, patients and health providers or authorities in order to increase the efficacy of diagnosis, treatment and follow up of patients with RA.

Design and Methods: The Project will be carried out by 48 partners covering the majority of MS. General methodology is designed on the basis of three transversal Work packages (WPs): three specific WPs for public health issues and management of patients with RAs and three additional WPs for Project's general management.

Results: Information and dissemination of knowledge, education and training, standardization patient's participation promotion and creation of an epidemiological registry for RAs.

SESSION 6: RED CELLS AND MALARIA

Oral communication #6.1

Implication of *Plasmodium falciparum* protein-kinase a in channel activation upon malaria infection

Stéphane Egée[1], Guillaume Bouyer[1], Anaïs Merckx[2], Gordon Langsley[3], and Serge Thomas[1], [1]CNRS-UPMC, Station Biologique, Roscoff, France, [2]Université Paris Descartes, Paris, France, [3]Institut Cochin-Université Paris Descartes, Paris, France

Objectives: Identification of mechanisms underlying up-regulation of new permeation pathways in *Plasmodium falciparum*-infected human red blood cells.

Design and methods: Whole-cell configuration of the patch-clamp technique was used on *P. falciparum* strain overexpressing protein kinase A regulatory subunit (PfPKA-R).

Results: We provide evidence that in *P. falciparum*-infected red blood cells, a cAMP pathway modulates anion conductance of erythrocyte membrane. In patch-clamp experiments performed on infected erythrocytes, addition of recombinant PfPKA-R to the pipette solution, or overexpression of PfPKA-R in transgenic parasites, lead to down-regulation of anion conductance. This PfPKA-R overexpressing strain has a growth defect that can be restored by increasing the levels of intracellular cAMP.

Oral communication #6.2

Glutathione-reductase deficiency. A new malaria-protective mutation?

Valentina Gallo[1#], Evelin Schwarzer[1#], Stefan Rahlfs[2], R. Heiner Schirmer[3], Dirk Roos[4], Paolo Arese[1], and Katja Becker[2], [1]Dipartimento di Genetica, Biologia e Biochimica, University of Torino, Torino, Italy, [2]Interdisziplinäres Forschungszentrum, Gieβen University, Gieβen, Germany; [3]Biochemie-Zentrum Heidelberg, Heidelberg University, Heidelberg, Germany, [4]Sanquin Research and Landsteiner Laboratory, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands. [#]Equal contribution

Objectives: RBC glutathione reductase (GR) regenerates GSH utilizing NADPH produced by G6PD. Mutations affecting both enzymes enhance oxidant sensitivity. Malaria-protective G6PD-deficiency is frequent while GR-deficiency is rare. We show that infected GR-and G6PD-deficient RBCs behaved similarly indicating that GR-deficiency may also be malaria-protective.

Design and methods: RBCs of three GR-deficient subjects were infected with *P. falciparum*. In isolated rings and trophozoites we analyzed: invasion/growth; drug sensitivity; deposition of heme/hemichromes, C3c complement-fragment, autologous-IgG; and ring/trophozoite-phagocytosis.

Results: Similarly to G6PD-deficient RBCs, parasite invasion/growth in GR-deficient RBCs was unimpaired. In ring-infected GR-deficient RBCs membrane deposition of heme, hemichromes and removal opsonin, and ring-phagocytosis were strongly increased. Based on the remarkable similarity to infected G6PD-deficient RBCs, GR-deficiency may thus add to the defense paradigm of other malaria protective genetic RBC mutations based on enhanced ring-stage phagocytosis rather than on impaired parasite invasion/growth. Supported by Regione Piemonte, RSF-project.

Oral communication #6.3

Role of hemozoin (HZ) and hz-generated 4-hydroxynonenal (HNE) in malaria dys-erythropoiesis

Oleksii Skorokhod, Paolo Arese, and Evelin Schwarzer, Dipartimento di Genetica, Biologia e Biochimica, University of Torino, Torino, Italy*

Objectives: To clarify the role of malaria pigment HZ and its product HNE in malaria dyserythropoiesis.

Design and Methods: Effects of HZ and HNE on proliferation, apoptosis and cell-cycle were studied in the erythroid cell-line K562 by FACS; transferrin receptor1(TfR1) was studied by qRT-PCR, WB and FACS.

Results: HZ generates lipoperoxides which decompose to HNE. After addition of HZ or HNE to cells, HNE-adducts on erythroid surface increased 3-fold and cell proliferation was reduced by 50%. HZ and HNE did not increase apoptosis and cell mortality. The cell-cycle was significantly altered. Dividing cells in the G2/M-phase decreased by 50% while G1-/S-phase-cells increased correspondingly. Cell-cycle changes decreased TfR1 expression, mediated by IRP2. Decreased TfR1 alters Fe-metabolism in erythroids, an effect possibly causally relevant for dyserythropoiesis in malaria. Supported by Compagnia di San Paolo and Regione Piemonte (Ricerca Sanitaria Finalizzata).

Oral communication #6.4

Effect of dihydroartemisinin (DHA) on human erythroid cell differentiation: Implications for malaria treatment in pregnancy

Sara Finaurini[1], Alessandra Colancecco[2], Luisa Ronzoni[2], Maria Domenica Cappellini[2], and Donatella Taramelli[1], [1]Department of Public Health, Microbiology-Virology, University of Milan, Milan, Italy, [2]Department of Internal Medicine, University of Milan, Fondazione Policlinico Mangiagalli, Regina Elena, IRCCS, Milano, Italy

Objectives: WHO does not recommend the use of Artemisinin Combination Therapy (ACT) to treat malaria during pregnancy, because animal studies showed a depletion of embryonic erythrocytes. We investigated the effect of Dihydroartemisinin (DHA), the metabolite of artemisinins, on an *in vitro* model reproducing human erythropoiesis.

Design and methods: $CD34^+$ cells differentiate towards erythroblasts under erythropoietin stimulus in 14 days. DHA, 0, 5 or 2 μM, was added on different erythroid stages. At different time cell growth, morphology, Glycophorin A expression as well as globin genes have been evaluated.

Results: DHA added on stem cells or on early progenitors caused a transient inhibitory effect, which was then fully restored. On the contrary, DHA added on more differentiated erythroblasts significantly blocked the erythroid differentiation. This indicates that DHA specifically affects the primitive erythropoiesis, occurring in the yolk sac. Therefore, during the first trimester of pregnancy, ACT must be avoided. EU Antimal Project 18834 is acknowledged.

Oral communication #6.5

Acute hemolytic crisis as a potential consequence of acetaminophen toxicity in a baby carrying g6pd-vanua lava mutation

Angelo Minucci, Paola Concolino, Palma Maurizi, Bruno Giardina, Cecilia Zuppi, and Ettore Capoluongo, Laboratory of Clinical Molecular Biology, Institute of Biochemistry and Clinical Biochemistry, Catholic University of Rome, Italy

Objectives: Although many drugs are well known as potential hemolytic triggers in G6PD deficient subjects, the paracetamol's (PCM) involvement is still discussed, even if an overdose of this drug can be responsible for acute haemolytic crisis (AHC). We present a case of an AHC occurred in a G6PD-deficient Philippine baby treated with PCM.

Design and methods: The patient came to our emergency department presenting with vomiting and fever. Laboratory results were compatible with AHC. Two blood transfusions and rehydratation treatment were done. For the genetic study, we analyzed *G6PD* gene and the common *SULT1A1* and *UGT1A1* polymorphisms involved in the altered PCM sulfonation and glucuronation.

Results: G6PD gene analysis revealed the presence of the *G6PD-Vanua Lava* mutation (*c.383T>C*). *SULT1A1* and *UGT1A1* were wild-type. Could the possible alteration of renal and hepatic PCM clearance or the hemoconcentration, due to the dehydration, be the cause of PCM toxicity in this patient? The question is opened.

List of posters

ERYTHROPOIESIS, IRON AND PORPHYRIAS

In search of a differential quotient to screen for HbE carriers

Chowdhury Prosanto, The Institute of Child Health, Kolkata, West Bengal, India

Efficacy and safety of deferasirox in multitransfused thalassaemics undergoing therapy for chronic hepatitis c and hiv infection

Prosanto Chowdhury, The Institute of Child Health, Kolkata, West Bengal, India

Identification of three novel substitutions in the erythroid promoter of the hydroxymethylbilane synthase gene (hmbs): a new erythroid variant of human acute intermittent porphyria (aip)?

Elena Di Pierro, Valentina Brancaleoni, Dario Tavazzi, and Maria Domenica Cappellini, Department of Internal Medicine, Fondazione Ospedale Maggiore Policlinico IRCCS, University of Milan, Italy

GENETIC DISORDERS OF THE RED CELL

Antioxidative enzyme activities in erythrocytes of β-thalassemia trait carriers: A preliminary study

Antonio Amato[1], Ofelia Sarra[1], Antonella Giuliano[2], Loredana Masi[1], Roberta Piscitelli[1], Alessandra Scardocci[1], and Giancarlo Ortaggi[2], [1]A.N.M.I. Onlus – Centro Studi Microcitemie di Roma, [2]Dipartimento di Chimica, Facoltà di SMFN, Università di Roma La Sapienza, Italy

Identification of hemoglobins with high oxygen affinity in sicily

Antonio Giambona, Margherita Vinciguerra, Cristina Passarello, Rita Li Muli, Filippo Leto, Germana Fiorentino, Filippo Cassarà, Monica Cannata, Pina Lo Gioco, Disma Renda, and Aurelio Maggio, Azienda Ospedaliera "V. Cervello", Unità Operativa di Ematologia II, Palermo, Italy

Hb benin city (β4 thr>pro): a new beta globin chain variant

Giovanni Ivaldi[1], Daniela Leone[1], Floriana Moruzzi[1], and Maura F. Musso[2], [1]Laboratorio di Genetica – Settore Microcitemia, Ospedali Galliera, Genova, Italy, [2]Laboratorio Analisi Chimico Cliniche e Microbiologiche, A.S.O. Santa Croce e Carle, Cuneo, Italy

Clinical, biochemical and molecular features of 507 patients affected by g6pd deficiency in Italy

Donatella Maffi[1], Francesco Sorrentino[2], Maria Teresa Pasquino[1], Maria Pia Caforio[1], Paolo Cianciulli[2], and Patrizia Caprari[1], [1]Istituto Superiore di Sanità, Dipartimento di Ematologia, Oncologia e Medicina Molecolare, Roma, Italy, [2]Ospedale S.Eugenio, Day Hospital Talassemie- Centro Anemie Congenite, Roma, Italy

Chronic hemolytic anemia due to pyruvate kinase (pk) deficiency in a patient heterozygous for haemoglobin s and no clinical features of sickle cell disease

Licínio Manco[1,2], J.M. Vagace[3], Luís Relvas[1], Umbelina Rebelo[1], Celeste Bento[1], and M. Letícia Ribeiro[1], [1]Departamento de Hematologia, Centro Hospitalar de Coimbra, Portugal, [2]Departamento de Antropologia/CIAS, Universidade de Coimbra, Portugal, [3]Servicio de Hematologia, Hospital Materno Infantil, Badajoz, Espanha

Discordant HUMARA and G6PD mRNA assays in a female with chronic haemolytic anaemia due to novel type i G6PD variant

Licínio Manco[1,2] Janet Pereira[2], Umbelina Rebelo[2], Celeste Bento[2], Jaspal Kaeda[2], and M. Letícia Ribeiro[2], [1]Departamento de Antropologia, Universidade de Coimbra, Portugal, [2]Unidade de Hematologia Molecular, Centro Hospitalar de Coimbra, Portugal

PK deficiency and mitral valve replacement: how to proceed?

Angelo Minucci[1], Bianca Maria Ricerca[2], Cecilia Zuppi[1], Bruno Giardina[1], and Ettore Capoluongo[1], [1]Laboratory of Clinical Molecular Biology, Institute of Biochemistry and Clinical Biochemistry, [2]Hematology Institute, Catholic University of Rome, Italy

G6PD pyrgos: A pathophysiological mechanism similar to that of G6PD A$^-$

Kamran Moradkhani, Claude Préhu, Henri Wajcman, and Frédéric Galactéros, INSERM Unité 955 and Hôpital Henri Mondor, Biochimie et Génétique, Créteil, France

Interlaboratory comparison of current high-performance methods for HbA_2

Andrea Mosca[1], Renata Paleari[1], Frederic Cotton[2], and Béatrice Gulbis[2], [1]Dip. Scienze e Tecnologie Biomediche, Università degli Studi di Milano, Italy; [2]Hôpital Erasme, Université Libre de Bruxelles, Bruxelles, Belgium

Association between duffy antigen expression and inflammation status in sca patients

Danitza Nebor[1], Marie-Claude Durpes[1], Danielle Mougenel[2], Jacques Elion[1], Marie-Dominique Hardy-Dessources[1], and Marc Romana[1], [1]Inserm, U763, Pointe-a-Pitre, Université des Antilles et de la Guyane, Pointe-à-Pitre, Guadeloupe, [2]Centre Caribeen de la Drepanocytose "Guy Merault", Centre Hospitalier Universitaire of Pointe-a-Pitre, Guadeloupe

MEMBRANE STRUCTURE AND STABILITY

Effect of temperature, different detergents and local anesthetics in the isolation of detergent-resistant membranes from human erythrocytes

Cleyton C. Domingues[1], Annarita Ciana[2], Bruna R. Casadei[1], Leonardo L. Souza[1], Cesare Balduini[2], Eneida de Paula[1], and Giampaolo Minetti[2], [1]Departamento de Bioquímica, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, SP, Brazil, [2]Università degli Studi di Pavia, Dipartimento di Biochimica, Pavia, Italy

Protective effect of phenolic compounds on hypercholesterolemia human erythrocytes

Piotr Duchnowicz[1], Anna Podsędek[2], Maria Koter-Michalak[1], and Marlena Broncel[3], [1]Department of Environment Pollution Biophysics, University of Łódź, Łódź, Poland, [2]Institute of Technical Biochemistry, Faculty of Biotechnology and Food Sciences, Technical University of Łódź, Łódź, Poland, [3]Department of Clinical Pharmacology, Medical University of Łódź, Łódź, Poland

Identification of new functional targets of AMPKα1 in mouse red cells

Andrea Biondani [1], Benoit Viollet[2,3], Marc Foretz[2, 3] , Carlo Laudanna[4, 5], Jocelyne Devin-Leclerc [2, 3] Giovanni Scardoni[4], Lucia De Franceschi[1], [1]University of Verona, Verona, Italy; [2]Institut Cochin, Universite' Paris Descartes Centre National de la Recherche Scientifique (UMR8104), Paris, France; [3]INSERM U567, Paris, France; [4]Department of Pathology, Section of General Pathology, and [5]CBMC- The Center for Biomedical Computing, University of Verona; Italy

Chloride conductance generated in human erythrocytes exposed to low extracellular ph and high osmotic pressure

Yuliya V. Kucherenko[1,2], [1]Department of Physiology, University of Tübingen, Germany, [2]Department of Cryobiophysics, Institute for Problems of Cryobiology and Cryomedicine of the Ukrainian National Academy of Sciences, Kharkov, Ukraine

Whole-cell patch-clamp study of the age-dependent cation channels activity in human RBCs

Yuliya V. Kucherenko[1,2], [1]Department of Physiology, University of Tübingen, Germany, [2]Department of Cryobiophysics, Institute for Problems of Cryobiology and Cryomedicine of the Ukrainian National Academy of Sciences, Kharkov, Ukraine

Indication of protein poor GM1-patches in the human erythrocyte membrane. does love for curves make you DRM?

Lucyna Mrówczyńska[1], and Henry Hägerstrand[2], [1]Department of Cell Biology, A. Mickiewicz University, Poznań, Poland, [2]Department of Biology, Åbo Akademi University, Åbo-Turku, Finland

Photosensitized hemolysis study: Lipid peroxidation leads to osmotic lysis of erythrocyte

Igor Olegovich Pivovarov[1], Alexandra Evgenyevna Kornienko[1], Anna Vladimirovna Valdohina[4], and Fazly Inoyatovich Ataullakhanov[1-3], [1]Moscow State University, School of Physics, Dept. of Biophysics, Moscow, Russia, [2]Center for Theoretical Problems of Physicochemical Pharmacology RAS, Moscow, Russia, [3]Research Center for Hematology RAMS, Moscow, Russia, [4]I.M. Sechenov Moscow Medical Academy, Moscow, Russia

Comparative analysis of mammalian erythrocyte resistance to mechanical stress

Nataliya Shpakova, Darya Aleksandrova, and Nataliya Orlova, Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkov, Ukraine

Membrane budding and vesiculation as indicators of health and disease

Vid Šuštar[1], Mojca Frank[2], Vid Janša[1], Nataša Resnik[3], Apolonija Bedina Zavec[4], Rado Janša[5], Blaž Rozman[2], Peter Veranič[3], Henry Hagerstrand[6], Aleš Iglič[7], and Veronika Kralj-Iglič[1], [1]Laboratory of Clinical Biophysics, Faculty of Medicine, University of Ljubljana, Slovenia, [2]Department of Rheumatology, University Medical Centre Ljubljana, [3]Institute of Cell Biology,

Faculty of Medicine, University of Ljubljana, [4]National Institute of Chemistry, Ljubljana, [5]Department of Gastroenterology, University Medical Centre Ljubljana, [6]Department of Biology, Abo Akademi University, Abo, Finland, [7]Laboratory of Physics, Faculty of Electrical Engineering, University of Ljubljana

Human erythrocyte thermotolerance inhibition by iodoacetamide

Michał Szewczyk, Piotr Duchnowicz, and Maria Koter-Michalak, Department of Biophysics of Environment Pollution, University of Łódź, Łódź, Poland

MEMBRANE TRANSPORT

Effect of circulating free heme on plasma arginase activity and L-arginine cellular influx

Lucia Cortelezzi, Zeuda Vommaro, Diletta Scaccabarozzi, and Fausta Omodeo-Salè, Institute of General Physiology and Biochemistry, "G.Esposito", University of Milano, Italy

METABOLISM

Diagnostic of the erythrocyte membranes' damages induced by electron beam of medical accelerator

Bliznyuk Uliana A, Chernyaev AP, The Scientific Research Institute of Nuclear Physics, Moscow, URSS

Research of antioxidants and muscle relaxants action on red cell membrane by the method of calibrated electroporation

P.Yu. Borschegovskaya, and V.V. Moroz, Moscow Reanimatology Research Institute, Moscow, Russia

The next step to the new method of red blood cell protection – viscoelasticity properties

Małgorzata Dzik[1], Ludmiła Słowińska[2], and Anna Marcinkowska-Gapińska[3], [1]Wrocław University of Technology, Institute of Biomedical Engineering and Instrumentation, Wrocław, Poland, [2]Medical University of Silesia, Department of Biophysics, Rheological Laboratory, Zabrze-Rokitnica, Poland, [3]Poznan University of Medical Sciences, Department of Neurology, Rheological Laboratory, Poznan, Poland

Do the changes of plasma viscosity modify RBC's aggregability?

Małgorzata Dzik[1], Anna Marcinkowska-Gapińska[2], and Małgorzata Komorowska[1], [1]Institute of Biomedical Engineering and Instrumentation, Wrocław University of Technology, Wrocław, Poland, [2] Department of Neurology- Rheological Laboratory, Poznan University of Medical Sciences, Poznan, Poland

The activity of Na^+K^+atpase in erythrocytes from patients after cryotherapy

Agnieszka Staroń[1], Grzegorz Mąkosa[2], Piotr Duchnowicz[1], and Maria Koter-Michalak[1], [1]Department of Environment Pollution Biophysics, University of Łódź, Łódź, Poland, [2]Department of Rehabilitation, Hospital in Tuszyn, Tuszyn, Poland

The antioxidant enzymes activity in erythrocytes from patients after cryotherapy

Agnieszka Staroń[1], Grzegorz Mąkosa[2], Piotr Duchnowicz[1], and Maria Koter-Michalak[1], [1]Department of Environment Pollution Biophysics, University of Łódź, Łódź, Poland, [2]Department of Rehabilitation, Hospital in Tuszyn, Tuszyn, Poland

The damage of erythrocytes of patients with metabolic syndrome

Anna Ziobro[1], Piotr Duchnowicz[1], Anna Mulik[1], Maria Koter-Michalak[1], and Marlena Broncel[2], [1]Department of Environment Pollution Biophysics, University of Łódź, Łódź, Poland, [2]Department of Clinical Pharmacology, Medical University of Łódź, Łódź, Poland

NOVEL TECHNOLOGY

Diagnosis of red cell membrane protein abnormalities in italian patients: Role of flow cytometric test and SDS page electrophoresis

Elena D'Alcamo, Veronica Agrigento, Serena Sclafani, Aurelio Maggio, Angela Vitrano, and Paolo Rigano, A.O. V. Cervello, U.O.C. Ematologia II con Talassemia, Palermo, Italy

www.tosohemoglobine.it, A new instrument of expertise for the diagnosis of thalassemia and hemoglobinopathies

Stefano Frediani, and Paola Bellati, Tosoh Bioscience, Italy

The investigation of structural inhomogeneities of erythrocyte surface by the atomic force microscope

Regina M.Rysaeva[1], and G.R. Kasiev[2], [1]Physical Department, Lomonosov's *Moscow State University, Moscow, Russia, [2]33 Ostroumov*'s *Moscow Municipal Hospital, Moscow, Russia*

Blood protection against oxidative stress

Tomasz Walski, Weronika Berlik, Izabela Synal, Karolina Grzeszczuk, and Małgorzata Komorowska, Institute of Biomedical Engineering and Instrumentation, Wroclaw University of Technology, Poland

RED CELL AND MALARIA

PCR-based kit for the screening and typing of malaria infections

Maurizio Gramegna[1], Alessia Moiana[1], Luana Coltella[2], Cristina Russo[2], and Simonetta Gatti[3], [1]Sentinel CH SpA, Milano, Italy, [2]Microbiology Unit- Laboratory Department Bambino Gesù Children Hospital- Health Care and Research Institute, Rome, Italy, [3]Laboratory of Parasitology, Virology Service – Foundation-IRCCS Policlinico San Matteo, Pavia, Italy