**Red Cell Special Interest Group 2017 – Report for Bloodlines**

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The Red Cell SIG meeting provides an annual forum for presentation of UK based red cell research and should be of interest to clinicians, haematologists, biochemists, or anyone involved in red cell research or wishing to learn more about red cells. The Red Cell SIG is usually held on the first day of the BBTS Annual Scientific Meeting, aiming to encourage participation by both the BBTS members and by the wider scientific community. This year, Red Cell SIG was held on Wednesday, 13th September, at the BBTS Annual Scientific Meeting in Glasgow. The meeting comprised three sessions, each with three speakers presenting the very latest developments in our understanding of red cell defects, physiology/function and malarial invasion.

**Session 1: Red Cell Defects**

The first speaker of this session, Anita Hill from the Department of Haematology at St James' Institute of Oncology in Leeds, gave an update on the diagnosis and management of primary autoimmune haemolytic anaemia (AIHA). Anita’s talk was centred on the new British Society for Haematology guidelines which aim to assist the diagnostic approach to suspected AIHA. The causes of haemolysis were described, which can be intrinsic, extrinsic and congenital/acquired, leading to either warm type or cold type AIHA, based on their typical serological characteristics. AIHA can be classified into primary (50% of cases) and secondary AIHA associated with other conditions (infections, foreign travel, surgical procedures). Anita proceeded to discuss the presentation and treatment options through an account of one especially difficult case study. The options for controlling the disease with minimal side effects were discussed, and different lines of therapy described: first line with prednisolone, second line with rituximab and failing those, the third line of therapy with splenectomy and either azathioprine, cyclosporin, danazol, or mycophenolate mofetil. The talk raised the question of side effects, such as osteoporosis, that can be devastating, especially in young patients.

The next talk on CRISPR/Cas9 editing of the major alpha-globin regulatory element as a curative strategy for beta-thalassaemia was given by Mohsin Badat from Weatherall Institute of Molecular Medicine, Oxford. This work focused on β-thalassaemia, the most common single gene disorder in the world. There are approximately 1,000 patients with the severe form of β-thalassaemia in the UK, and 60,000 severely affected children born each year worldwide. In β-thalassaemia, the disrupted balance between α and β globin chains caused by the inactivating mutations in *HBB* gene lead to accumulation of α globin, ineffective erythropoiesis and ultimately haemolysis and anaemia. Mohsin has used powerful CRISPR/Cas9 gene editing, which, in his words, has a potential to alter the course of human history, to alter the ratio of α and β globin by disrupting the key regulating element MCS-R2 of α globin locus. The regulator was deleted in primary human haematopoietic progenitor cells which differentiated into erythroid cells with the reduction in α-globin expression, delivering a correction of the pathologic globin chain imbalance and opening the possibility of future refinement of the method, and ultimately, gene therapy.

The third speaker of the morning session was Mark Layton from the Department of Haematology, Imperial College London. Mark introduced the AG-348 pyruvate kinase activator and its effects on anaemia and haemolysis in patients with pyruvate kinase (PK) deficiency. PK deficiency is a rare genetic disease that reduces glycolysis and therefore the production of ATP causing chronic haemolytic anaemia. AG-348 is an allosteric activator of PK that restores glycolysis and therefore the red cell ATP levels in patients with PK deficiency. Mark discussed updated findings of the DRIVE PK open-label, global phase 2 study, conducted in 14 centres in US, Canada and Europe. Initially, a total of 52 patients were recruited (mostly men, majority splenectomised) and randomized to two arms of the study with AG-348 50 mg or 300 mg administered orally. Of the patients tested during the core period of the study, 48% had a rapid and sustained maximal increase in haemoglobin >1 g/dL seen across the range of doses. Genotype–response correlations was observed, where 57% patients had at least one missense mutation in PK gene and in turn haemoglobin >1 g/dL increase. AG-348 was generally well tolerated. Five patients had serious adverse events, including drug-induced anaemia, osteoporosis, hypertriglyceridaemia and pharyngitis, but the most common adverse events were insomnia, headache and nausea. AG-348 has proved to be a first-in-class PK activator in clinical testing with potential as a disease-altering therapy for patients with PK deficiency.

**Session 2: Red Cell Physiology / Function**

José González-Alonso, Department of Life Sciences, Brunel University London, opened this session with his talk on red cell temperature sensitive mechanisms and their relevance for blood flow control during hyperthermia. José introduced the hypothesis that the ATP release from red cells is sensitive to physiological increases in temperature, providing a mechanism of regulating muscle and skin perfusion in conditions that alter blood and tissue temperature. Local hyperthermia increases blood temperature, brachial artery blood flow and plasma ATP levels. Infusion of ATP into the brachial artery increases deep tissue perfusion similarly. *In vitro*, in isolated red cells exposed to different temperatures ATP release increased at higher temperatures and declined at lower temperatures. No changes in ATP levels were seen in cultured human endothelial cells, plasma or serum samples under the same conditions, suggesting that red cells are responsible for the temperature-sensitive release of ATP. The mechanisms of ATP release from red cells are thought to involve membrane-bound ion channels (band 3, band 4.5), gap junction proteins (pannexin 1), and ATP-binding cassette proteins (ABC proteins). Blocking of band 3 appears to prevent hyperthermia-induced ATP release. Future research will elucidate whether red cell temperature sensitive signalling plays an important physiological role in the vascular and haemodynamic adaptations to heat therapy in patients with circulatory disorders.

The second talk of the session ‘Cation leakage from red cells (CARED) study of familial pseudohyperkalaemia’ was presented by Helen New, Consultant in Paediatric Transfusion Medicine, NHSBT, London on behalf of the CARED study group. The CARED study was set up to study the effect of familial pseudohyperkalaemia (FP) on stored red cells. In stored standard red cell units potassium levels in the bag steadily increase over the 35 days of cold-storage. For this reason it is advised that red cell packs for neonatal and infant large volume transfusion (LVT) should be stored for no more than 5 days, to reduce the risk of hyperkalaemia following transfusion. However, in stored FP red cell units potassium levels increase much more rapidly, and a day 5 unit may contain up to 57 mM supernatant potassium making it potentially unsuitable for use in situations where hyperkalaemia may be a risk. FP is a dominantly inherited, asymptomatic condition caused by a non-synonymous single nucleotide polymorphism in the *ABCB6* gene with a population frequency of 1:400. The CARED study undertook screening of the NIHR Cambridge Bioresource to identify FP individuals. Out of 8712 individuals screened, 16 were identified as FP SNP carriers, whilst two more were identified in clinical cases. Six FP individuals in total were recruited for whole blood storage studies. A slight change of cell volume was observed in stored FP red cells relative to controls, and the increased rate of potassium release from red cells was confirmed in all FP units stored under standard NHSBT conditions. Whether, in the future, it will be possible to systematically genotype these donors to prevent the risk of hyperkalaemia in situations such as infant LVT was discussed.

In the last talk of the session, Mike Murphy, NHSBT, Oxford University Hospitals and University of Oxford, gave an update on the blood group antigen matching influence on gestational outcomes (AMIGO) study. The study was conducted across 14 centres in North America, Europe and Australia, exploring the effectiveness of a policy for blood group antigen matching in decreasing the risk of alloimmunisation. The study was designed to examine the proportion of haemolytic disease of the newborn (HDFN) due to maternal sensitisation from transfusion, which was usually related to obstetric haemorrhage. Two types of centres were included; those that routinely match for red cell antigens and those that do not. The results have shown that most HDFN is due to previous pregnancy (85% in ‘no match’ and 82% in ‘match’ centres) and only 3% of HDFN was due to transfusion. Most common antibodies involved in alloimmunisation were Rh, Kell, Wra and Jkb. No direct protective influence of ‘match’ centre policies was observed. Mike suggested that global antigen matching transfusion policies may improve results, but the use of antigen matching in localised areas only may not provide an impact.

**Session 3: Malaria Research**

The last session of this year’s Red Cell SIG started with a talk ’Inhibition of an erythrocyte tyrosine kinase with imatinib prevents *Plasmodium falciparum* egress and terminates parasitaemia’, given by Franco Turrini, University of Torino, Italy. Currently, malaria affects approximately 188 million people, and although numbers of deaths are reducing in the last 15 years, drug resistance is increasing. Franco has investigated the use of tyrosine kinase inhibitors that target a human gene, not under parasite control, making it more difficult for the parasite to develop resistance. Red cell membrane protein band 3 stabilises the red cell by connecting to the spectrin/actin cytoskeleton via an association with ankyrin. Tyrosine phosphorylation of band 3 causes its dissociation from ankyrin, membrane destabilization, vesiculation and haemolysis. In malaria, band 3 becomes a target for binding of tyrosine kinase (syk) that phosphorylates band 3 and allows *P. falciparum* egress via increased vesiculation and eventual cell rupture. To inhibit this process, Franco’s group has treated *in vitro* the malaria infected cell cultures with Imatinib, a tyrosine kinase inhibitor that has known activity against syk. The treatment was very promising, blocking phosphorylation of band 3, preventing parasite egress and decreasing malarial parasitaemia in cell cultures.

The malaria theme continued with a talk by Marion Koch, Department of Life Sciences, Imperial College London, about dissecting the mechanics of erythrocyte invasion by the malaria parasite *P. falciparum*. The mechanics of red blood cell invasion by *P falciparum* merozoite is a process which lasts only 30 seconds. The merozoite orients itself with its apical side to the cell and uses reticulocyte-binding-like protein homologues (RH) and erythrocyte binding antigens (EBA140, 175, 181, etc.) to directly bind different receptors on the surface of the red cell and eventually engage the actin-myosin motor. In *P. falciparum*, most EBA proteins interact with erythrocyte glycophorin proteins. EBA175/glycophorin A (GPA) interaction can cause decreased deformability of the red cell due to increased cross-linking of the C-terminal domain of GPA to cytoskeleton. Marion has used flicker spectroscopy and real time deformability cytometry assays to assess membrane tension and deformability, respectively. When EBA175 is added to red cell membranes, tension was significantly increased. Using naturally occurring Miltenberger red cells, that lack the C-terminal domain of GPA, the membrane tension dropped, whilst the bending modulus showed the same trend. Decrease in the bending modulus would have increased parasite efficiency. Marion is planning to investigate further the effect of cholesterol in red cell membranes may affect *P falciparum* invasion efficiency.

The last talk in this session was given by Gavin Band from Wellcome Trust Centre for Human Genetics, University of Oxford, who presented his work on the resistance to malaria through structural variation of red blood cell invasion receptors. He introduced the evolutionary battle between *P.falciparum* and the red cell and explained how a genome-wide association study was conducted on 17,000 severely affected children and controls from 11 endemic populations in Africa. There are already a number of red cell variants known to provide some protection against the malaria (blood group O, sickle cell disease trait, G6PD deficiency, etc.). However, the team has identified a region on chromosome 4 near the glycophorin genes, which appeared to be correlated with severe malaria status. The team has used whole genome sequencing (WGS) to build a panel of genetic variants across the glycophorin region, followed by inheritance studies and Sanger sequencing to confirm the accuracy of the WGS. They used a statistical approach to identify structural variants in all 3,269 sequenced samples and identified 16 variants in total, with two of those being identified as the Dantu variant of the MNS blood group system. In West Africa the frequency of Dantu is low but in East Africa it is much higher. Using meta-analysis, they undertook a study across the Gambia, Kenya and Malawi assessing severely affected, hospitalised population and healthy controls and discovered that people with Dantu phenotype had a 40 per cent reduced risk of severe malaria.

The 2017 Red Cell SIG meeting was well attended, generating many questions and encouraging audience participation and discussion.