Training Course on Red Blood Cells: Genesis and Pathophysiology

Chantilly, France. April 23-26, 2014

Erythropoiesis, the process by which CD34+ cells (the precursors of red blood cells) mature and acquire their definitive characteristics, occurs at distinct sites in the organism. These processes are affected by the environment surrounding the cell, called the erythroid niches: different environments present at different stages of developments that drive to the generation of slightly different mature red blood cells. The characteristics of the niche, still not fully understood, should guarantee the proliferation and maturation of red blood cells at the rate required by the organism, and have to assure the pass through the different stages of maturation:

- The commitment of the progenitor to the erythroid lineage
- The proliferative phase
- The differentiation of the cells, controlled externally due to erythropoietin.

During these stages, different molecules are required, some of them well characterized like Epo (Erythropoietin) and SCF (Stem Cell Factor), and also used for cell culture systems. Epo, for example, is specifically required for as to guarantee the proliferation and maturation of cells, and also the protection from apoptosis. The amount and sensitivity of immature red blood cells to Epo will determine, together with the Fas/FasL system, the amount of cells that will undergo maturation or apoptosis: this system of regulation will guarantee the maturation of the correct number of red blood cells.

Different characteristics of the mature red blood cell are achieved during differentiation:

- Iron incorporation in the maturing cell, necessary for oxygen loading. The process of iron loading in cells is tightly regulated by hepcidin and erythroferrone, among other hormones. Failure of the process of iron loading can lead to anemia or hemochromatosis (iron defect or accumulation in the organism).
- Removal of intracellular organelles and cytoplasmic material, by autophagy of all the material, during terminal differentiation, in mammalian erythrocytes. Each intracellular organelle is eliminated in different moments of maturation, with an up regulation of the process in the polychromatophilic stage.

Some factors as hypoxia or anemia can alter the regulation of the maturation process, and induce the generation of an increased number of mature red blood cells. In the case of hypoxia, the transcription factor
HIF regulates erythropoiesis. Hypoxia, also when caused by altitude, can induce a chronic excessive number of red blood cells, causing pulmonary hypertension and high hemoglobin concentration.

**Red blood cell diseases**

Malaria is one of the major causes of death in tropical and subtropical area, where it is endemic, and is caused by the infection of red blood cells by different species of *Plasmodium*, being *Plasmodium falciparum* the most deadly form. During the symptomatic phase of the disease the parasite infects erythrocytes and undergoes intensive multiplication, during cycles of 48hour. During this time, parasites induce an intensive remodeling of the red blood cell, including installing organelles, changing the permeability of the host cell membrane and inducing cell adhesive properties. All this remodeling is set up by the parasite to guarantee the supply of energy necessary for the rapid rate of nucleic acid synthesis and protein synthesis. The membrane remodeling, changing the adhesive properties, permit the parasite to adhere to vital organs and to the endothelium, and escape from clearance by the spleen. Iron supply is required in a high quantity by the parasite, in fact, low access to iron of the parasites was suggested to permit and increase of the phagocytosis of parasitized red blood cells.

Sickle cell anemia or SCD, which is due to a mutation in the hemoglobin gene, has a variable clinical course, with different life threatening conditions. The disease can be modified by varying levels of different proteins, like HbF (fetal hemoglobin). Different haplotypes of the HbS gene (the modified hemoglobin gene) can determine various outcomes, and new strategies for gene identifying, like GWAS (Genome wide association studies) would be necessary to find new genes and genome regions susceptible to predispose to or modify the development of SCD.
Meeting report: Training Course on Red Blood Cells, genesis and pathophysiology
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There were two major learning objectives to this meeting, one related to diagnosis and treatment of red cell disorders, and the other one related to developing high quality research projects on red cells. The following topics were addressed during this training:

- Intrinsic and extrinsic mechanisms that control red blood cell differentiation
- Metabolism of the mature red blood cell and control of cell shape
- Genetic diseases affecting red cell functions
- Acquired abnormalities of the red cell

This meeting was divided into five sessions, the first too concern the normal erythropoiesis. Differents topics were discussed. V. Betin presented that removal of organelles and cytoplasmic material during terminal differentiation of mammalian erythrocytes is an essential and highly regulated process involving autophagy.

S. Volon explained the importance of iron metabolism in erythropoiesis, indeed the principal function of erythrocytes is to deliver oxygen from the lungs to the other tissues of the body. To perform their duty as oxygen carriers, erythrocytes require iron. Iron is so important that all deregulation leads to a more or less severe disease such as anemia. Many study are performed on hepcidin appears to be a good therapeutic target.

P. Ratcliffe, J.P. Richalet and J.F. Toussaint explained role of oxygen sensing and hypoxia signaling pathways. Erythropoietin is a hormone essential for erythropoiesis. It is induced in excess of a thousand-fold in severe anaemia and to a lesser extent by small changes in blood oxygen availability, such as occur after phlebotomy or on ascent to modest altitude. Studying of this signaling allows better understanding chronic mountain sickness or Monge’s disease.
which characterized by an excessive number of red cells in the blood of persons living permanently above the altitude of 2.500m and other pathologies.

The last three sessions were introduced by L. Douay who wants to do large-scale erythroid differentiation using iPS cells. The objective in the medium term of producing red blood cells aimed at transfusion is to provide patients under particular circumstances of transfusional shortage i.e. patients presenting a rare blood group or those that are polytransfused and alloimmunized (sickle cell disease, dysmyelopoiesis), as novel therapeutic solution.

The three sessions focused on diseases of red blood cells. The fourth focused specially on inherited erythropoiesis disorders with the Diamond Blackfan Aneamia presented by L. Da Costa. DBA is a congenital erythroid aplasia that usually presents in infancy and DBA patients have low red blood cell counts (anemia). The rest of their blood cells (the platelets and the white blood cells) are normal. This pathologies is heterogenous, but also genotype. This gene described was the ribosomal protein RPS19 identified in 25% of DBA cases. A mutation in RP gene is responsible for a defect in rRNA maturation and ribosome biogenesis. Franconi anaemia, congenital dyserythropoietic anaemia and telomereopathies were presented respectively by J. Soulier, A. Lolascon and I.S. Dokal.

The fifth session introduced by S.Vaulon exposed acquired anaemia and especially paroxysmal nocturnal hemoglobinuria presented by R. Peffault De Latour. This pathology is a rare disorder of hematopoietic stem cells and the disease is diagnosed with hemolytic anaemia, marrow failure or episodes of venous thrombosis. PNH is related to a somatic mutation in the phosphatidilinositol Glycan class A, X-linked gene, responsible for a deficiency in glycosyl phosphatidilinositol-anchored proteins. The lack of one of the GPI-AP complement regulatory proteins CD59 leads hemolysis. Recently inhibition the complement cascade with Esculizumab, which is a C5-inhibitor humanized monoclonal antibody, reduced intra vascular hemolysis, leading to improved anaemia with reduced transfusion and increased transfusion independence. The fifth session that ends with a presentation of 5 clinical cases presented by A.P Azevedo, M. Berenguer-Piqueras, M. Sanchez and J.L. Vives-Corrans to better understand acquired anaemia.

The last session was intended to inform on Malaria and sickle cell disease and was exposed by A. Merckx and H. Drakesmith. The protozoan disease is transmitted from one person to another by female mosquitoes of the genus Anopheles, and is caused by five parasite species that affect humans: Plasmodium falciparum, P. vivax, P. ovale, P. malariae and P. falciparum. During the symptomatic phase of the disease that occurs within the erythrocytes, the parasite undergoes intensive multiplication, which requires a rapid rate of nucleic acid synthesis and derived proteins are expressed, exported and presented at the surface of the infected erythrocyte membrane and change cell permeability. However, the widespread use of desferrioxamine (Fe3+ chelator) was limited owing to its poor membrane permeability and absorption when given orally, a short half-life in plasma, and a slow-to-develop antimalarial effect. The development of antimalarial drugs that target the parasite’s access
to iron might meet with more success if we had a better understanding of how Plasmodium parasites acquire iron during their various life stages.

The last part of the conference was presented by F. Piel, L de Franceschi and J. Elion. They exposed sickle cell disease. Sickle haemoglobin results from a single amino acid substitution at position 6 of the beta globin molecule. This mutation occurs on five classical haplotypes and these haplotypes were first identified by the presence or absence of restriction fragment length polymorphisms and subsequently found to be characterized by strong linkage disequilibrium. Initially, the quest of gene modifiers has been undertaken via a candidate gene approach, the first of which being the b-globin gene cluster on chromosome 11. But the results were not sufficient to treat the disease.

To conclude, the training course on Red Blood Cells, that has been my first convention, allowed me to better understand the functioning of red blood cells at physiological and pathological level and approach the different therapeutic perspective to treat these multiple pathologies.