

Current Understanding of the Red Blood Cell Diseases

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The human red blood cell is produced continuously from hematopoietic stem cells in the bone marrow. These hematopoietic stem cells can differentiate into red blood cell precursors, which continuously produce hemoglobin until it accounts for roughly 90% of the dry cell weight. The red blood cell precursors then undergo enucleation, a process in which the nucleus of the precursor cell is squeezed out to generate a mature red blood cell.¹ The mature red blood cell takes the rather unique shape of a biconcave disk, which is maintained by structure proteins in the cell membrane. The life span of a mature erythroblast is roughly 120 days in humans. A variety of red blood cell diseases are known, such as anemia, sickle cell diseases, malaria, hemolysis and polycythemias. Diseases involving red blood cells are generally due to three factors: genetic deficiency, poor nutrition or parasitic infection. In the past years, progress in genetic and molecular research tools has allowed for a deeper understanding of red blood cell diseases. In this paper, we'll provide a review of advances in understanding and treatment of the most common red blood cell diseases: namely Malaria, Sickle Cell Disease, and Iron Deficiency Anemia.

Malaria

Malaria is caused by the protozoans of the genus *Plasmodium*. *Plasmodium falciparum* is most common infectious agent of malaria in humans, and is also responsible for the most malaria related mortalities. The emergence of drug resistant strains of *Plasmodium falciparum* has been a major obstacle in controlling this disease. Recently, a number of new drugs based on artemisinin derivatives have been shown to be highly effective.² The World Health Organization now recommends artemisinin based drugs, combined with traditional antimalaria amines as the therapy of choice to treat malaria infection.³ Again, the specter of drug resistance looms on the horizon. Artemisinin functions by disabling a vital calcium pump in *Plasmodium*, and recent research have shown that the mutation of a single amino acid is sufficient to confer resistance.⁴ Fortunately, the complete

genomic sequence of *Plasmodium falciparum* was published in 2002, and various genomic and proteomic maps have since been proposed, giving significantly more tools to malaria drug researchers.^{5,6}

A malaria vaccine remains the holy grail of malaria research. Historically, vaccination was the most effective method to contain infectious diseases. Despite intensive efforts in the past two decades, no effective vaccine is currently in clinical use.⁷ The development of vaccine has been complicated by the fact that the disease agent in malaria is protozoan as oppose to the traditionally common bacterial or viral agents. The most promising vaccination target in recent years is the circumsporozoite protein (CSP) of *Plasmodium falciparum*.⁸ This protein is critical for sporozoite function and invasion of red blood cells.⁹ RTS,S is a CSP vaccine that combines CSP antigen with a surface antigen from hepatitis B, which leads to a more potent immunogenic response.¹⁰ The RTS,S vaccine with AS02A adjuvant was shown to give 34% protection in adults and 30% protection in children.⁸ The vaccine is expected to be released in 2011.

Sickle Cell Disease

Sickle cell Disease (SCD) has been studied for nearly 100 years. The genetic and molecular cause of this disease was first elucidated in 1950s. SCD is an autosomal recessive, genetically inherited blood disorder that is characterized by sickle shaped red blood cells in affected patients. The shape is due to a single point mutation in the beta-globin chain of hemoglobin, which leads to a propensity for the hemoglobin to aggregate and distort the shape of the blood cell.¹¹ This disease is mostly seen in descendants of populations in which malaria is or was prevalent. Heterozygosity of Sickle Cell gene is known to confer partial immunity to malaria.¹² In SCD patients, the "sickled" red blood cells have decreased flexibility and mobility.¹³ This leads to a number of complication and risk factors, including vasco-occlusive crisis, spleen enlargement, stroke, gallstones, jaundice and more. Patients with sickle cell disease have a life expectancy of 42 for males and 48 for females.¹⁴

A number of different strategies have been employed for the treatment of sickle cell anemia. In one method, the fetal hemoglobin is reactivated epigenetically to replace the defective hemoglobin in SCD patients. The first drug that utilizes this mechanism is hydroxyurea, which was shown to be effective at increasing the life expectancy of patients.¹⁵ Because hydroxyurea is a chemotherapeutic agent, there remain concerns that this drug can be potentially harmful in

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the long term. Some recent studies focused on finding alternative methods to induce the expression of fetal hemoglobin. Butyrate is a short chain fatty acid that inhibits histone deacetylase. This then leads to acetylation and upregulation of the fetal hemoglobin. When a variant of butyrate is administered to patients, it resulted in sustained fetal hemoglobin expression in SCD patients.¹⁶ However, butyrate has to be administered through central venous catheters, and this delivery limitation poses major challenge to the use of butyrate as a common treatment.¹¹ Another way to increase fetal hemoglobin production is through DNA hypomethylation. Previous studies have suggested that the expression of fetal hemoglobin or adult hemoglobin is controlled through DNA methylation.¹⁷ Decitabine is a recently studied compound that has shown great potential in inducing fetal hemoglobin expression through DNA hypomethylation.^{18,19} Small scale clinical studies have shown that this drug is highly effective and significantly reduced the risk factors associated with SCD.¹⁹

Bone marrow transplantation is so far the only curative therapy for SCD patients.²⁰ This treatment replaces the bone marrow, which is the source of the faulty hemoglobin with bone marrows that produce normal red blood cells. However, bone marrow transplantation therapy is limited by the availability of matching donors. Recent studies on mouse models have shown that hematopoietic precursors obtained from induced pluripotent stem cells (ISP) can be used to treat and cure SCD in mice.²¹ Of course, ISP cells have their own problems with oncogenesis that needs to be resolved before this therapy is suitable for clinical use. Fortunately, progress is swift on this front.

Gene therapy provides another potential curative therapy for SCD. A number of studies in the past decade have shown that this technique is therapeutically viable.²² However, the standard caveats for gene therapy also applies in the treatment of SCD, such as delivery difficulties, long term effectiveness and oncogenic concerns.

Iron Deficiency Anemia

Iron deficiency is often considered the world's single most prevalent nutrition deficiency.²³ Chronic and severe iron deficiency often leads to iron deficiency anemia (IDA), in which the hemoglobin of the Red Blood Cell cannot form due to a lack of iron. Iron deficiency can be caused of a wide variety of reasons. It often occurs in otherwise healthy females due to menstrual blood loss.²³ In pregnant woman, the growth of the fetus and expanded blood volume drastically increase the body's iron requirement, and often, the body's iron supply simply cannot keep up.²³ In developing countries in which parasitic infections are prevalent, the infection of hookworms or whipworms almost inevitably leads to IDA.^{24,25} In one study, it is estimated that 35% of IDA cases in children are directly linked to hookworm infection.²⁶ The economic cost of hookworm induced IDA is enormous. IDA as a result of hookworm infection may cost up to 5 billion dollars a year due to lost productivity.²³

Furthermore, IDA has been shown to correlate with poor motor, cognitive development in infants, as well as impaired language, motor, and cognitive performance in toddlers and young children.²⁷ General symptoms of IDA include sleepiness, fainting, esophageal web, hair loss, and other symptoms common to anemia. IDA in pregnant woman can be especially dangerous, and potentially fatal.²³

The standard treatment of IDA in adults is oral intake of 300 mg of ferrous sulfate three times daily. Ferrous Iron is thought to be easier for the body to absorb due to its solubility and availability at the pH of the digestive tract. However, a recent study has suggested that intravenous iron sucrose treatment provides a better efficacy and lower side effects.²⁸ Some researchers are currently advocating the combination of erythropoietin and intravenous iron sucrose therapy to rapidly reverse the effect of anemia.²⁹

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