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Editorial

A Brief Note on Human Red Blood Cell Proteome

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EDITORIAL NOTE

RBCs are the most common form of circulating human cell and are necessary for blood gas transport. Although proteins on the RBC surface can influence illness susceptibility, prior research that classified the RBC proteome did not apply specialised methodologies for concentrating cell surface proteins. Furthermore, no comprehensive study of variation in RBC surface protein abundance among genetically diverse human groups has been conducted. These topics are crucial not only for understanding fundamental RBC biology, but also for identifying novel malarial parasite potential receptors. The spectrum of clinically recognised blood types, from the main Rhesus grouping to the more minor Landsteiner-Weiner system, is defined by inter-individual variance in red blood cell surface proteins. Understanding the diversity of RBC surface proteins is crucial for understanding transfusion compatibility across donors and recipients, as well as disorders such infant haemolytic illness.

Proteins on the surface of red blood cells are also important in determining susceptibility to blood-borne illnesses. For example, an increasing number of proteins are recognised as receptors for Plasmodium species, causative agents of malaria. The antigens of the Duffy blood group system were once thought to be *Plasmodium vivax* receptors. Additional molecules have recently been discovered as *Plasmodium vivax* or *Plasmodium falciparum* species receptors. Basigin, complement receptor 1 (CR1/CD35), CD55 (complement decay-accelerating factor/Cromer blood group), CD44 (Indian blood group), glycophorin molecules, the Langereis blood group protein ABCB6, and Transferrin receptor are only a few examples. Selection against Duffy antigens has been driven by long-term selective pressures from infections such as Plasmodium species in malaria-endemic populations.

All internal cell structures are absent in mature red blood cells, which are made up entirely of cytoplasm enclosed by a plasma membrane envelope. Total red blood cell protein was separated into two fractions i.e membrane-associated proteins and cytoplasmic proteins, in order to achieve the best results. Both fractions were split into subfractions, and tryptic digestion was used to identify proteins in each fraction separately. Externally exposed proteins, internally exposed proteins, spectrin extract, and membrane proteins without spectrin extract were all used to make membrane protein digests.

Several earlier investigations of the red blood proteome have been conducted, but none have focused primarily on proteins exposed at the cell surface. Proteomic analysis of other less abundant proteins has been more difficult due to the fact that haemoglobin subunits and carbonic anhydrase make up over 98 percent of the protein content of RBC. Various approaches, ranging from isolation of 'white ghost' membranes to absorption of the highly abundant haemoglobin prior to analysis, have been utilised to selectively enhance these proteins for proteomics. Despite this, these methods are still contaminated by a large number of intracellular proteins and provide only a limited understanding of which proteins have extracellular domains. In order to separate fresh RBC for proteome analysis with optimum purity and yield while minimising *ex vivo* modification, a simple approach was designed.

Previously, enrichment methods included a combination of strategies to deplete leukocytes (cellulose, density gradient or simple centrifugation, and negative selection using magnetic beads), remove platelets (PBS washing, and positive erythrocyte selection using magnetic beads) and remove plasma constituents (cellulose, density gradient or simple centrifugation, and negative selection using magnetic beads), (simple centrifugation and PBS washing). Flow cytometry was used as readout to quantify residual contamination with platelets and leukocytes, and a variety of these approaches were compared. The contamination of serum components was then evaluated using a sensitive proteomic method. Centrifugation of 2 × 1 ml anticoagulated blood at 1000 g for 5 minutes was optimised, followed by extraction of 150 µl packed RBC from the very bottom of each Eppendorf tube. The cells were resuspended in PBS, filtered with a Plasmodipur filter, and washed twice.

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