Flow Cytometric Evaluation of Red Blood Cells Transformed with Variable Amounts of Synthetic A and B Glycolipids

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Background

According to national guidelines or directives, monoclonal ABO reagents may be required to detect A, and Bweak subgroup red blood cells (RBCs). Many routine laboratories do not have access to naturally-occurring ABO subgroups that can be used as weak controls for these reagents. Group O RBCs modified with synthetic analogs of blood group A and/or B glycolipids (KODE™ technology) to mimic weak ABO subgroups could be used for quality control purposes.

Aim of the Study

Extensive serological testing of KODE™ RBCs has previously been carried out. An extended evaluation of KODE™ RBCs using flow cytometry was performed to explore the correlation between the concentrations of synthetic glycolipids and A/B antigen site density of the resulting RBCs. The aim of this study was to examine if KODE™ RBCs mimic the distinct flow cytometric patterns of naturally-occurring ABO subgroups and to identify the optimal concentration of glycolipid required.

Materials & Methods

Samples: KODE™ RBCs were prepared according to a previously described procedure. The RBCs were modified with 15 different concentrations of synthetic glycolipids, ranging from 1000 µg/mL to 0.06 µg/mL for KODE™A and 5000 µg/mL to 0.3 µg/mL for KODE™B. The concentration was decreased by doubling dilution steps. For both KODE™A and KODE™B RBCs, repeat samples were produced for four selected concentrations as a consistency measurement and all KODE™ batches were tested in triplicate.

Flow Cytometry: Sensitive and specific flow cytometry was used to characterize and semiquantify the synthetic A and B antigen levels on group O RBCs. Relevant control RBCs (A, A-, A, B, Bweak and O) were included in each run. Primary antibodies: Anti-A (ES-15, Serologicals Limited, West Lothian, UK) Anti-B (9621A8, Diagast, France). Secondary antibody: PE-labelled rat-anti-mouse Ig kappa light chain (Becton Dickinson, CA, USA).

Results

Flow cytometric testing of KODE™ RBCs modified with high concentrations of synthetic glycolipids revealed a uniform and even distribution of antigens in the cell population as shown by a single narrow peak in the FACS histograms. When KODE™ cells that resembled the naturally-occurring subgroup control RBCs in the population. The concentrations of synthetic glycolipids which produced A and B subgroups indicating a more variable antigen site density on the cells. This is in contrast to naturally-occurring subgroups in which some cells express almost no A or B antigen whilst others have close to normal levels. The reason for this is unknown. KODE™ RBCs obviously lack A/B-carrying glycoproteins but it is not fully understood to what extent glycolipid versus glycoprotein A/B epitopes contribute to the phenotype of weak subgroups. This study indicates that KODE™ RBCs with weak expression of A and/or B antigen have characteristics compatible with use as quality controls for monoclonal ABO reagents and could be a valuable addition in the serological laboratory.

References

1. Frame et al., Synthetic glycolipid modification of red blood cell membranes. Transfusion 2007;47:876-82
2. Hult & Olsson ML. Genetically defined ABO subgroups exhibit distinct flow cytometric patterns. Transfusion 2006;46:35A