Background

The provision of suitable red cell blood components for transfusion and pregnancy can be complicated by the presence of serologically complex red cell antibodies. Recombinant blood group proteins have been shown to have potential in the elucidation of red cell antibodies. Recombinant blood group proteins are available in numerous forms; soluble recombinant proteins that can be used for antibody inhibition, solid phase immobilised recombinant proteins and protein micro-arrays or colour coded microspheres.

Aims

The study primarily evaluated the use of cell culture supernatant (CSN), as a source of unpurified recombinant protein in haemagglutination inhibition assays, thus facilitating the identification or exclusion of underlying allo-antibodies. The use of CSN eliminates the protein purification step during recombinant protein production.

Methods

Antibodies to blood group antigens K, k, Kp, Js, Fy, Fy, and Lu were inhibited using the following recombinant proteins: 3xFLAG-K, 3xFLAG-k, Fy3xFLAG, Fy3xFLAG and Lu3xFLAG protein (K and k proteins also express Kp and Js). Recombinant proteins were analysed in two forms; 1) CSN containing soluble recombinant protein (CSN-SRP) and 2) purified protein product for comparison. Recombinant proteins were provided by Diagnostics Development, NHSBT Filton, with funding from BIORAD. CSN-SRPs (of unknown concentration) and purified recombinant proteins (of known concentration) were assessed for their ability to inhibit known clinically significant allo-antibodies. All antibodies tested (n=41) were titred to determine their strength and the strongest samples were selected for inhibition. Antibody isotype (IgG/IgM) was also determined by BIORAD monoclonal DAT. Antibody identification was performed by BIORAD IAT pre- and post-inhibition. Both patient and reagent antibodies were tested.

Results

CSN-SRP was extremely effective at inhibiting all patient antibodies tested (titre range from 1 to 512). Samples with an IgM component required a higher volume of CSN-SRP to completely inhibit the antibody. A ratio of 1:10 (v/v) CSN-SRP to plasma, incubated for 10 minutes at 37°C was the optimum method for antibody inhibition. All antibodies were inhibited with CSN-SRP with the exception of one reagent monoclonal anti-K (IgM). The inhibition of selected allo-antibodies facilitated easier identification of other underlying allo-antibodies, especially where multiple antibodies or antibodies to high frequency antigens were present (Table 1, Figures 1, 2, 3 & 4).

Conclusions

Successful antibody inhibition using purified protein has been previously reported. This study shows that CSN-SRP is an acceptable alternative eliminating the need for protein purification. The relatively small volume of CSN required for inhibition prevents dilution of other antibodies present in the sample, allowing the effective detection or exclusion of other underlying antibodies. A ratio of 1:10 (v/v) CSN-SRP to plasma, incubated for 10 minutes at 37°C was the optimum method for antibody inhibition.

References


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