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Original Article

Isolation, Cloning, Expression and Purification of Recombinant RhD Antigen from Cord Blood

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Abstract:

Background: Rh (Rhesus) is a highly complex blood group system in man deeply rooted in transfusion medicine. Isolation of RhD from cord blod, cloning and expression of recombinant RhD antigen in bacterial expression system was the aim of this study.

Methods: Total RNAs were extracted from cord blood (O^+). The quality of RNA was determined by electrophoresis. In order to obtain coding sequence of RhD antigen cDNA was synthesized and Rh D gene was amplified by RT-PCR. The isolated RhD gene was cloned to pUC18 vector and transformed to *DH5α*. The confirmed construct was sub cloned into expression vector, pBADgIII/A, and expressed in *Top10 E.coli*. The expressed protein was characterized by SDS-PAGE and western blot analysis. Antigenicity of the expressed protein was assessed by ELISA using commercially available human anti-RhD polyclonal antibody with peroxidase conjugated goat anti-human IgG, IgM, IgA as secondary antibody.

Results: RhD gene was successfully cloned and expressed. The expected size of recombinant RhD protein was detected in SDS-PAGE, and confirmed by dot and western blot analysis. RhD antibody reacted with recombinant RhD antigen as well as with RhD polypeptide extracted from RBCs membrane.

Conclusion: The recombinant RhD may be helpful to further investigate the molecular basis of RhD protein and could be applicable for production anti- D antibody in an animal model.

Keywords:

Recombinant RhD antigen . *Cloning* . *Cord blood*

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