Expression of Rhesus D (RhD) antibody in transiently transformed tobacco plants.

A fast plant expression system for complex molecules

Marianne Dissing1, Leif Kofoed Nielsen2,Thomas Bouquin4, John Mundy3, Morten Hanefeld Dziegiel2, Carsten Meier1
1Aresa BioPharma, Symbion Fruebjergvej 3, DK-2100 Copenhagen Ø, Denmark
2H:S The Bloodbank, K12033, Copenhagen University Hospital, Blegdamsvej 9, DK-2100 Copenhagen Ø, Denmark
3Department of Plant physiology, Molecular Biology Institute, Copenhagen University, Ole Maaløes vej 5, DK-2200 Copenhagen, Denmark
4Maxygen, Agern alle 1, DK-2970 Hørsholm

Introduction

Monoclonal human anti Rhesus D IgG1antibodies are used in diagnostic tests in the identification of the Rhesus D+ RhD+ blood typing. Addition of these antibodies to RhD+ red blood cells results in agglutination or clumping of the blood cells. This phenomenon can be tested in gel cards where agglutinated cells are caught in the gel matrix and non-agglutinating cells are centrifuged to the bottom of the gel. Expression of recombinant protein by transiently transformed tobacco can be done in a few days and is a very low cost process. In order to investigate the potential of a transient transformation system of tobacco we used the human Rhesus D antibody (RhD ab) as a test candidate and compared the biological activity and usefulness in the diagnostics, compared to the antibody expressed in Chinese Hamster Ovary (CHO) cells.

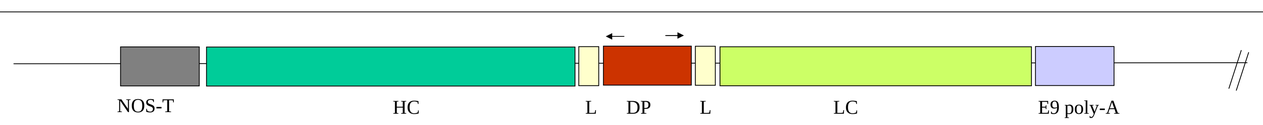

Fig 1. Essential features of the binary plasmid 3300 HC-LC used for plant trasformation. L; Murine leader signal sequence, LC: Varable and constant region of light chain, HC; variable and constant region of heavy chain, DP; mas1´2´ dual promoters with transcription in both directions, NosT; nopaline synthase transcriptional terminator, E9 poly-A; Pea rubisco small subunit transcriptional terminator (Bouquin, 2002).

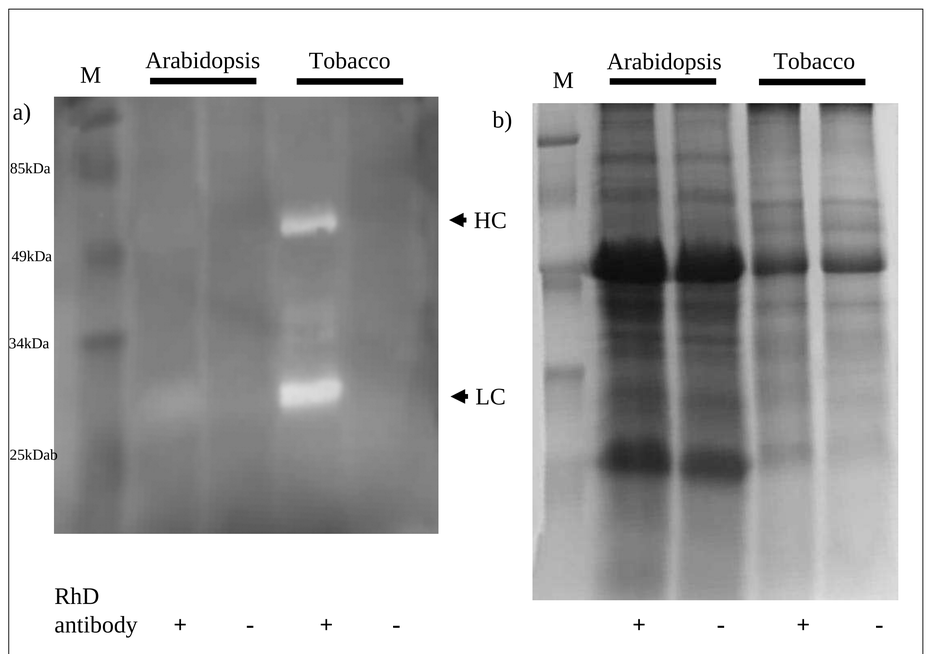

Fig 2. **Expression of RhD antibody in plants**.Total protein was extracted from transiently transformed tobacco and transgenic *Arabidopsis*, homozygous for the RhD antibody, and samples were analysed in parellel by a) Immuno blotting using anti human IgG antibody and b) Coomassie blue staining. Sampels from plants expressing recombinant RhD antibody are labelled RhD+. Wildtype Arabidopsis and tobacco plants used as controls are labelled RhD- . HC; heavy chain, LC; light chain and M; prestained protein standards.

Results

In order to investigate the ability of transiently transformed tobacco to express functional recombinant plantibody RhD ab we use the plasmid 3300 HC-LC, fig 1, which co-expresses the light and heavy RhDab cDNA sequence from a dual promoter. The plasmid was introduced into the plants by agro-mediated transformation of whole tobacco plants. In order to compare the antibody expression level in the different plant tissues total protein was extracted from the transformed tobacco leaves and also from *Arabidopsis* leaves of a transgenic RhD ab line generated with the same plasmid (Bouquin, 2002). As seen in fig 2, specific polypeptides at approximately 55kDa and 30kDa, interpreted as the antibody heavy and light chain polypeptides, was significantly higher expressed in the tobacco than in *Arabidopsis*. RhD plantibody was purified from transformed tobacco plants using protein G column chromatography and complete purification was determined by silver stained gel electrophoresis (not shown). The plantibody concentration was determined by ELISA and the ability to agglutinate RhD + red blood cells was compared to the CHO control antibody when dilution series of the antibodies were assayed by DiaMED anti IgG gel cards, fig 3. The results show that the plantibody can agglutinate RhD+ blood cells at a concentration down to 4 ng/ml fig 3 b). This is a little less, but similar in range to the CHO antibody which agglutinates red RhD+ blood cells at a concentration of 1.25ng/ml fig 3a). We think that the difference is due to a decreased concentration of functional antibody in the plantibody batch, caused by a very high pH (pH 9.6) of the elution buffer during the purification procedure. The specificity of the plantibody was analysed by testing the ability to agglutinate red blood cells from a panel of 20 blood donors with different blood types. As seen in fig 4 red blood cells were agglutinated from the 12 RhD+ donors and not from the 8 RhD - donors showing a high specificity against the RhD+ epitope.

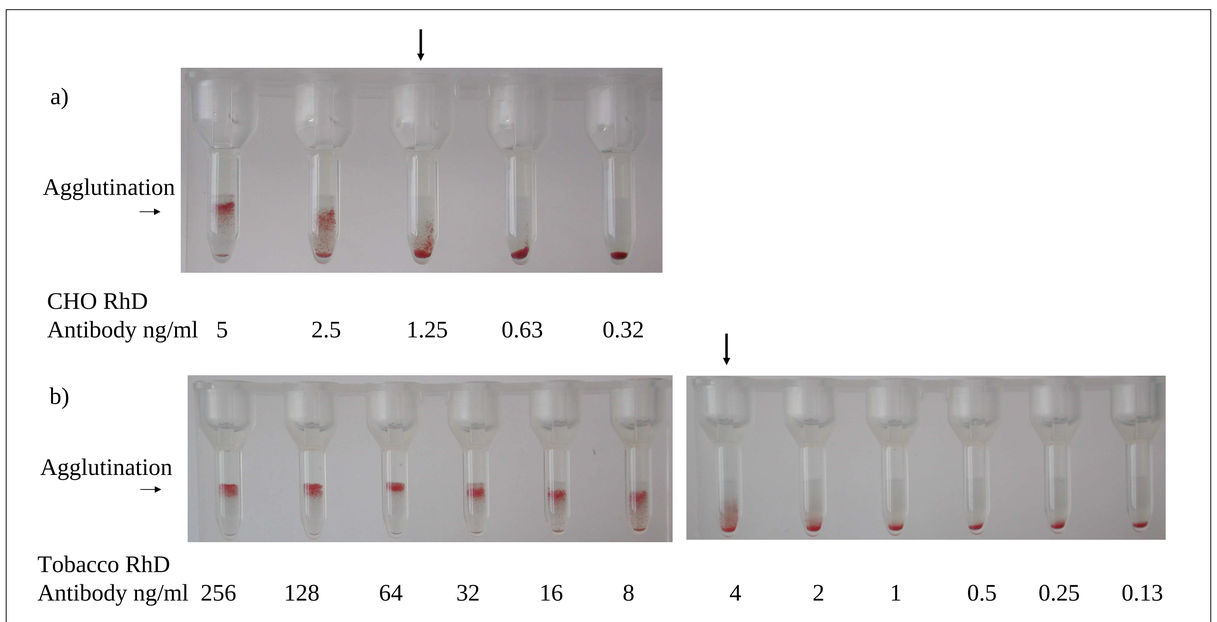

Fig 3. RhD antibody titration against RhD positive blood cells. DiaMED anti-IgG gelcards were used in the analysis. Delution series of antibody were mixed with RhD+ red blood cells and 75µl mixture were added to the gel card, incubated 15 min and centrifuged.The verical arrows indicate the limiting antibody concentration for agglutionation.

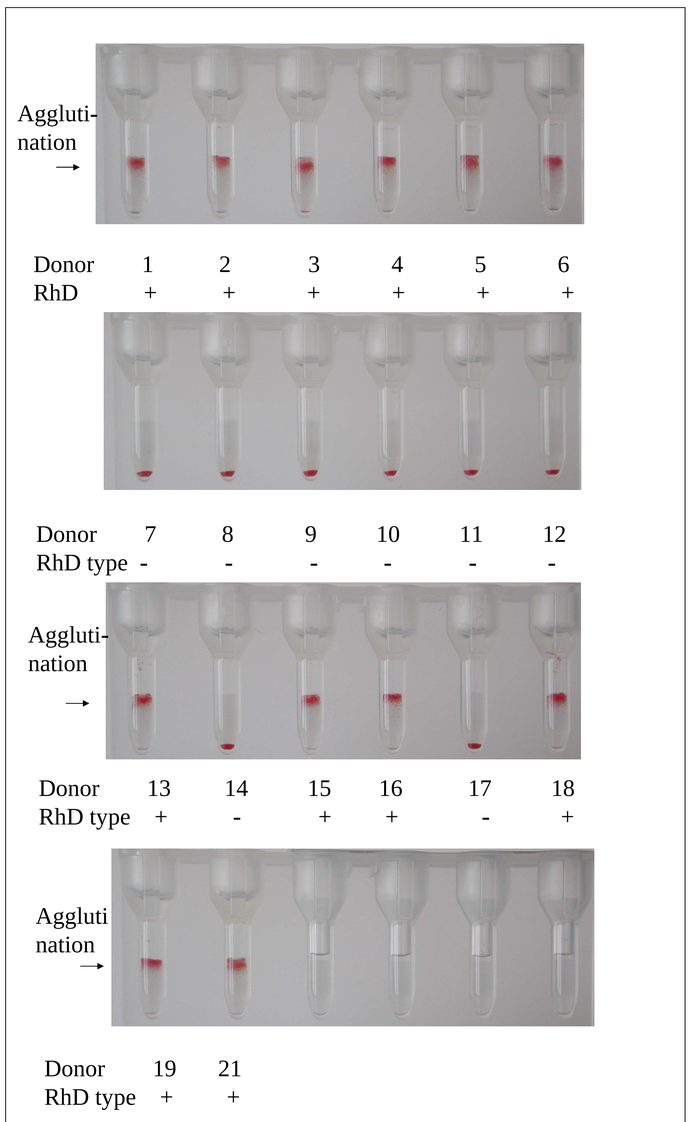

Fig 4. Tobacco RhD antibody specificity
Toccao RhD antibody were tested for specificity for RhD+ red blood cells against a panel blood donors, 12 RhD+ donors and 8 RhD- donors. Antibody were mixed with red blood cells from each donor and the analysis was carried out with DiaMED anti-IgG gel cards as described in fig 3.

Conclusion

Using a transient transformation assay in tobacco, the production and subsequent purification of the antibody can be done in less than two weeks. We tested the functionality of recombinant expressed RhD antibody in tobacco and found that the specificity of the antibody was just as good as recombinant RhD antibody produced in CHO cells. In addition the antibody functions at a similar concentration as the CHO in the agglutination assays in particular if we choose a different purification procedure.

Referaces: Bouquin et al, Transgenic research 11: 115-122, 2002