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Recombinant Soluble Blood Group Proteins: One Centre's Experience

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Background

The identification of allo-antibodies to high frequency antigens (HFAs) can be time consuming, even for national or international red cell reference laboratories. This can lead to delays in identification of the specificity of these antibodies and in the provision of suitable blood for these patients [Seltsam *et al.*, 2003].

Standard approaches for the identification of allo-antibodies to HFAs involve testing the patients's plasma against cells negative for various HFAs.

With all the major blood group genes cloned, the production of recombinant blood group proteins (rBGPs) to any protein-based blood group is possible. These rBGPs can be used in a soluble form to neutralise antibodies to HFAs [Seltsam *et al.*, 2014].

Aims

We investigated, both retrospectively and in-parallel, different patient plasma with known or suspected antibodies to HFAs. We used the Imusyn soluble recombinant blood group proteins (srBGPs). The srBGPs include the following blood groups: C4A, C4B, YTA, LUB, DOA, DOB, KNA, JMH, SC, CR.

Methods

Materials used:

•Plasma samples containing known antibodies to HFAs
•Patient plasmas tested in-parallel, containing suspected antibodies to HFAs
•Recombinant Soluble Blood Group Proteins (Imusyn)
•Bio-Rad LISS-Coombs Cards
•Bio-Rad Red Cell Panels

Patient plasma was neutralised with various srBGPs. 25µl of test plasma was incubated at 37ºC for 30 minutes with 2µl (1µg) of srBGP. The neutralised plasma was then tested by IAT using standard BioRad LISS-Coombs Cards and panel cells

Antibodies to HFAs Tested

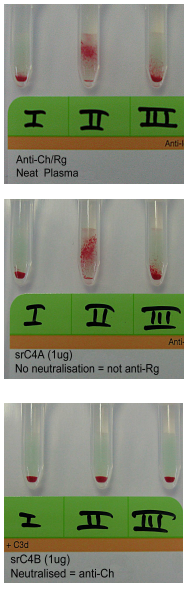
Retrospective	
Anti-Lu8	1
Anti-c+Jk^a+Yt^a	1
Anti-Yt^a	2
Anti-JMH	1
Anti-Lu^b	1
Anti-CR1-related + (?) anti-Fy^a	1
Anti-CR1-related	4
Anti- Ch/Rg	1
In-Parallel	
HFA antibodies	2
Anti-CR1-related	1
HTLA antibodies	5

Results

A total of 20 plasmas were tested. Retrospective testing was performed on 12 plasmas, while in-parallel testing was performed on 8 plasmas: Antibody to HFA (2), 'HTLA' antibody (5), anti-CR1-related (1).

Within the 'Retrospective' plasma group all antibodies were neutralised as expected except one anti-CR1-related. Anti-Lu8 was successfully neutralised by *srLUB* protein. One of the anti-Yt^a plasmas had an additional anti-K identified, following use of *srYTA*. The plasma containing anti-CR1-related with possible anti-Fy^a proved to contain just anti-CR1-related (during initial investigation all 'CR1' cells used were Fy(a-b-)).

Within the 'In-parallel' plasma group the 2 antibodies to HFAs proved to be O_h with anti-H (*srCR* and *srSC* were used but did not neutralise). A known anti-CR1-related from an African antenantal patient with an urgent blood request was neutralised by *srKNA* (compatibility testing was not performed with neutralised plasma). Of the 5 'HTLA' antibodies investigated 3 proved to be anti-CR1-related and neutralised with *srKNA*. Two of these anti-CR1-related plasmas were from African antenatal patients requiring urgent investigation. A third 'HTLA' antibody was identified as anti-Yt^a (*srYTA* successfully neutralised). The final HTLA antibody was tested with *srJMH*, which did not neutralise and was reported as 'weak undefined' (although further investigation identified the specificity as anti-Cs^a).



Differential identification of anti-Ch using srC4A and srC4B

Conclusion

Soluble rBGPs proved very useful in investigating the specificity of antibodies to high frequency antigens. In particular *srKNA* proved very useful in neutralising anti-CR1-related in antenatal African patients requiring urgent investigation and provision of suitable blood, rapidly eliminating the possibility of anti-Fy3. The Imusyn srBGPs have recently received CE-marking and would be an extremely valuable and useful addition to the repertoire of tests available to any laboratory investigating difficult serological samples, especially antibodies to HFAs.

References

Seltsam A, Wagner F, Lambert M, Bullock T, Thornton N, Scharberg EA, Grueger D, Schneeweiss C, Blasczyk R. Recombinant blood group proteins facilitate the detection of alloantibodies to high-prevalence antigens and reveal underlying antibodies: results of an international study. *Transfusion* 2014;**54**:1823-1830.

Seltsam A, Wagner FF, Salama A, Flegel WA. Antibodies to high-frequency antigens may decrease the quality of transfusion support: an observational study. *Transfusion* 2003;**43**:1563-1566.

Additional Data

Since submitting this abstract our laboratory has used the recombinant soluble proteins on two more occasions.
•A plasma reactive with C4-coated cells but did not neutralise with pooled AB serum. Neutralisation was achieved with 3µg of srC4B, revealing the presence of anti-K. On subsequent referrals Ch- cells were used to exclude the presence of other alloantibodies.
•Antenatal patient with anti-D (>1000 IU/ml), anti-C and anti-E following several IUTs, appeared to have developed an antibody to a HFA. We were able to rule out several by using srLUB, srYTA, srDOA, srDOB, srJMH, srCROM, srSC. We also typed the patient's cells for several HFAs and used several HFA- cells. Finally, after referral to IBGRL, the antibody was identified as an Rh-specific auto-antibody.

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