PROJECT REVIEW

"Immunorecognition of specific markers of blood storage. Customization and testing of already developed antibodies. (Acronym: Hemab2)"

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There is evidence that changes occur in red blood cells (RBCs) stored ex-vivo (e.g. in a blood bag or as a frozen concentrate) that do not occur in a normal RBC population. The underlying research proposal follows-up the empirical selection of recombinant antibodies able to selective recognize blood samples having been stored using regular procedures (i.e. bags of RBC concentrates kept at 4ºC or frozen) already performed as part of a first year granted project (HemAb). Our team successfully selected several clones from a large phage display antibody library that selectively recognize stored blood.

To complete the Proof of Concept phase of the project we propose to do as follows:

1.- Optimization of the selected clones

Especially we will increase the valency of the recombinant antibodies from the current monovalent stated. In addition we will examine various tag-sequences to identify the optimal tag, considering the final detection system and last we will optimize the affinity of the most ideal recombinant antibody if needed.

2.- Testing of the different clones in Flow Cytometry conditions with blood samples under different storage conditions.

- Fresh blood samples
- Blood samples stored at 4ºC > 30 days (including kinetic samples taken along the storage period)
- Blood samples stored frozen >30 days

3.- Optimization of the FC methodology

Fluorophores, combination of antibodies, pre-cell enrichment, etc.

4.- Development and validation of the scale-up production of the selected clones. To ensure that the assay can be transferred to partner laboratories large scale production of the most optimal recombinant antibody will be optimized and a production strain will be frozen.

5.- Testing of real samples of transfused individuals.

- Blood samples from transfused patients (homologous, HBT)
- Blood samples from transfused volunteers (autologous, ABT)

RESULTS AND CONCLUSIONS

AIMS

The main objective of the project was the development of recombinant monoclonal antibodies able to recognize changes in red blood cells (RBCs) specifically related to storage conditions (blood bags). The antibodies will be used to develop a method to unambiguously identify the use of blood transfusion, either autologous (ABT) or homologous (HBT). This is a follow-up project.

RESULTS

Using Phage Display, a series of 133 different clones were selected showing some selectivity towards recognizing stored RCs with respect to freshly collected RBCs.

Two different selection procedures and two different testing procedures were assayed along the selection procedures, i.e. ELISA and/or Flow Cytometry.

After testing those selected clones under different conditions, trying to expose and recognise the RBC storage-specific antigens, both surface or cytoplasmic, one of them was able to selectively detect stored RBCs in the presence of a vast excess of fresh RBCs.

CONCLUSIONS

Despite initial results showing the selection of some promising clones able to selectively differentiate stored RBCs from fresh RBCs, none of the clones worked properly under Flow Cytometry conditions in any experimental set-up tested. New rounds of selection were made without fruitful results.

FUTURE PLANS

Phage Display continues being a very powerful tool to find proper antibodies able to detect changes in RBCs under storage conditions. However, so far, our selection attempts have not been fruitful. Literature indicates that other changes are produced in RBCs upon ex-vivo storage (E.g. proteasome changes) for which proper antibodies could be developed.

Consideration will be given to all those potential changes and the appropriate set-up needed to improve the feasibility of such development in the near future.