“Direct detection of homologous and autologous blood transfusion through immunorecognition of specific markers of blood storage. (Acronym: Hemab)”

Dr. J. Pascual, Dr. P. Kristensen, Dr. C. de Bolos (Fundació IMIM, Spain)

Project Overview:

Blood transfusion is a prohibited method in sport. The currently applied method to detect homologous blood transfusion is based on the detection of cell surface blood group antigens that would be different between donors. There is no method available to detect autologous blood transfusion. However, 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 present proposal intends to empirically select antibodies from a large phage display antibody library able to recognize those specific changes in stored RBCs. Using the novel technology of single cell selection, antibodies recognizing only stored cells will be selected and against antigens broadly occurring in stored cells and that do not show in naturally aging RBCs. Thus, the objectives of the project are:

1.- Selection of an antibody(s) clone from a phage display Library able to specifically identify red blood cells which have been stored in a blood bag or frozen and reconstituted as red blood cell concentrate.

2.- Study the kinetic evolution of the detected antigen along storage period or blood manipulation.

3.- Produce recombinant antibody formats containing the proper tag for optimal use for cell sorting technologies (MACS, FACS)

4.- Development of a Magnetic-activated cell sorting (MACS) procedure for single cell detection.

5.- Adaptation of the methodology to a Flow-Cytometry platform for higher throughput.

Results and Conclusions:

AIMS

The main objective of the project was to the develop of antibodies able to recognize changes in red blood cells (RBCs) related to ex-vivo storage to unambiguously identify the use of blood transfusion, both autologous blood transfusion (ABT) or homologous blood transfusion (HBT).

RESULTS

Eight promising clones (single domain antibodies fragments, sdAb) were selected showing potential application as a screening and/or confirmation test to selectively recognise stored RBCs.

On the other hand, flow cytometry has proven to be sensitive enough to detect 0.1% cells of a target population and, using a pre-concentration technique like cell sorting, this limit of detection can be greatly increased.

Once the sdAb fragments have been selected from the phage display library, they can be optimised by modifying each position in the CDR regions to increase specificity, optimise the tag sequences with which they are expressed, etc. so that the final instrumental set-up is optimised.

CONCLUSIONS

The overall results demonstrate that the selection of antibodies from a large phage display library is a feasible approach to develop a procedure to detect surface antigens produced on RBCs through ex-vivo blood storage. These antigens can be potentially detected using flow cytometry in a minute subpopulation of RBCs in a freshly drawn blood sample.