No alloantibodies against mesenchymal stromal cells, but presence of anti-fetal calf serum antibodies, after transplantation in allogeneic hematopoietic stem cell recipients

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Supplementary Figures

Figure 1

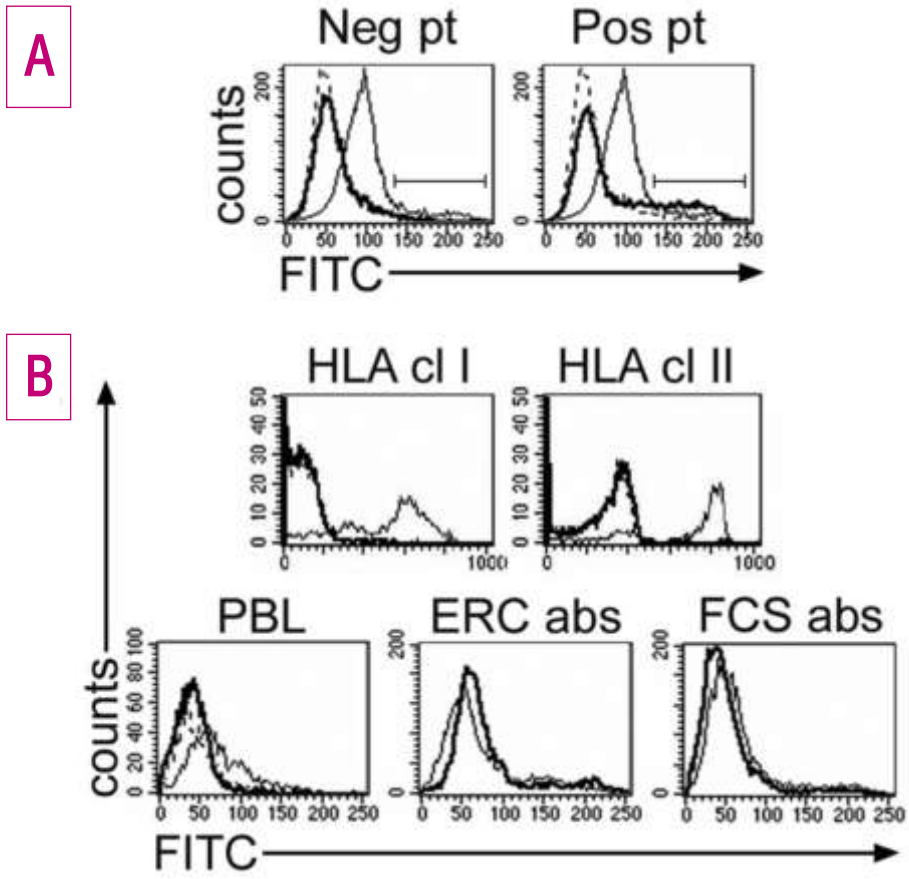

Figure 1. Antibodies causing positive flow cytometric cross matches using mesenchymal stromal cells are directed against fetal calf serum. A. Representative flow cytometric cross matches (FCXM) in one negative and one positive patient. Dashed lines indicate negative control, thin lines represent the positive control and the bold line denotes the patient sample. The marker indicates the where the negative and positive results differ. B. No anti-HLA antibodies were found in patients that developed a positive FCXM 9-12 months post-transplant. Top panels show representative Flow-PRA® analyses.

Dashed and thin lines denote negative and positive control, respectively. Bold line represents the patient sample. The bottom panel shows a, representative, specificity determination of the antibodies causing the positive reactions. No reactivity against peripheral blood lymphocytes (PBL) was detected (denoted as FCXM). After absorption with erythrocytes (ERC abs) no difference in mean fluorescence was seen, but after absorption with fetal calf sera (FCS abs) the mean fluorescence was reduced and FCXM regarded negative. Thin lines and bold lines indicate the patient sample from before and after absorption, respectively.

Figure 2

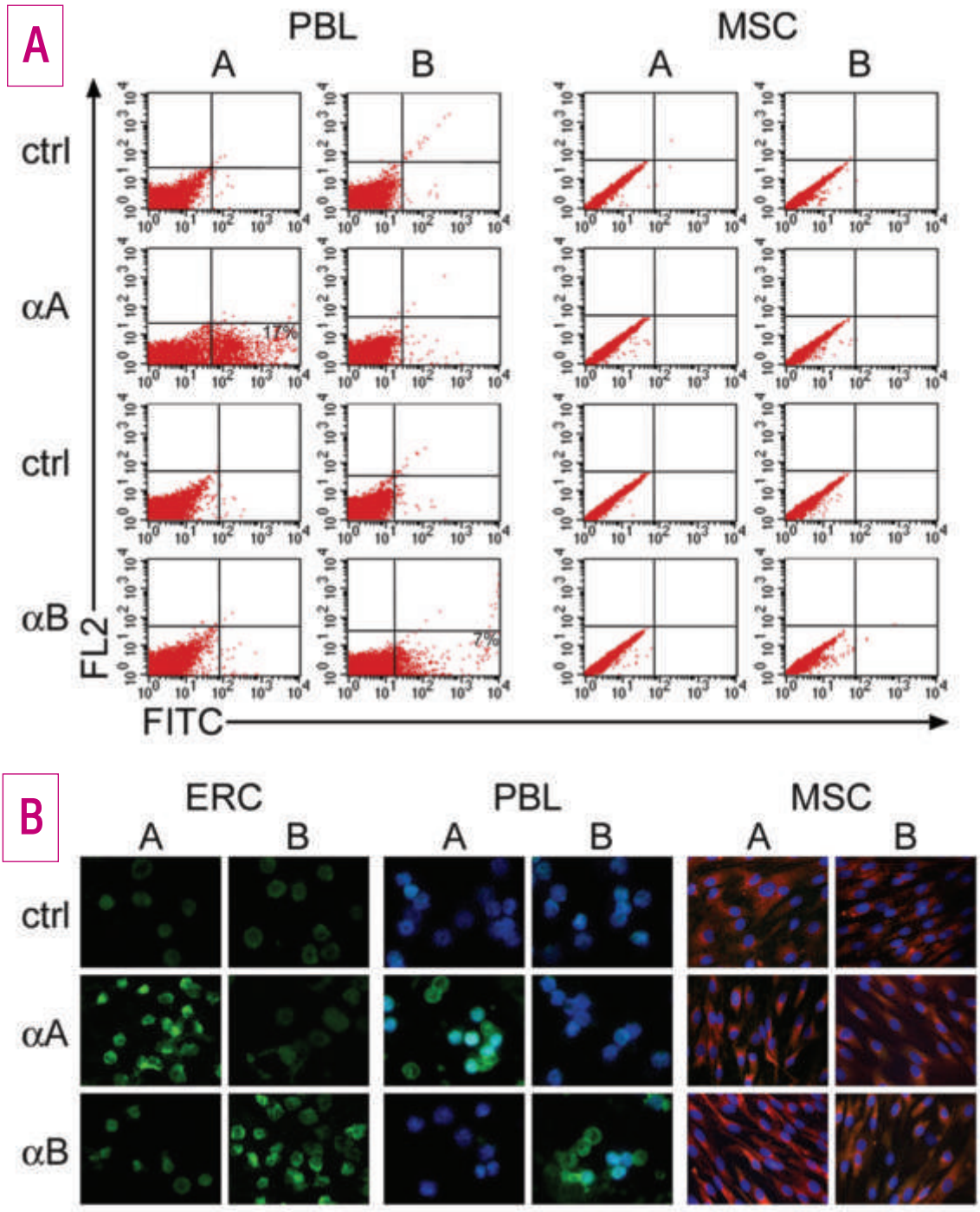

Figure 2. No expression of blood group A and B antigens were seen on mesenchymal stromal cells. A. Representative flow cytometric analyses of blood group antigen expression on peripheral blood lymphocytes (PBL) and mesenchymal stromal cells (MSC) from blood group A and B donors, respectively. Crtl represents cells incubated with the fluorescinated secondary antibody and αA/αB represents cells incubated with both the primary and secondary antibodies, respectively. Percentages of positive cells are indicated, when positivity was found. The PBL A and PBL B were positive for blood group A and B antigens, respectively. No expression on MSC could be demonstrated. B. Erythrocytes (ERC) expressed high levels of blood group antigens, PBL low to intermediate levels and MSC were negative in the immunofluorescence assay. Denotation with crtl and αA/αB are used as above.

Figure 3

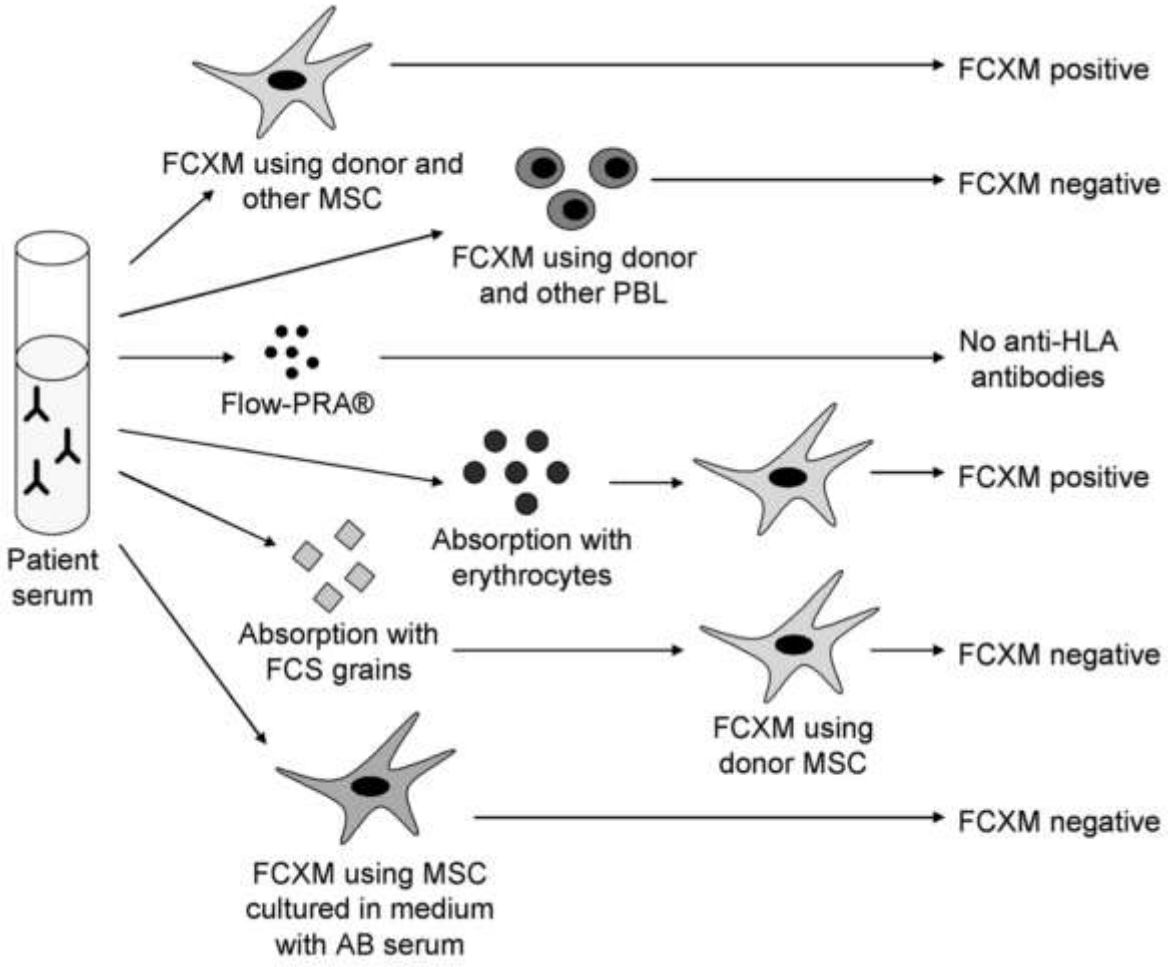

Figure 3. Specificity determination of antibodies causing positive flow cytometric cross matches (FCXM) post-transplantation of mesenchymal stromal cells (MSC). Sera found positive in the FCXM using donor MSC were negative if incubated with donor PBL, but remained positive if incubated with MSC derived from other donors. No anti-HLA antibodies were detected in the Flow-PRA®, further indicating that no alloimmunization had occurred. The positivity was not due to antibodies against blood group antigens as sera were still positive after absorption with erythrocytes. Finally, the sera were negative after absorption with fetal calf serum (FCS) grains. Similarly, the sera were negative if incubated with MSC generated in AB serum instead of FCS.