Biotin-kodecytes - novel Function-Spacer-Lipid (FSL) modified cells capable of being recovered from the circulation after 3 days

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Background

The ability to modify a population of blood cells with both an antigen of interest and an identification-recovery label, infuse them into the circulation of an animal, and then visualize or recover a sample of the infused cells some hours-days later for analysis, is now possible through the use of FSL (function-spacer-lipid) constructs.

FSL constructs are analogous in structure to a flower and consist of three components; a **F**unctional head group (like the flower head), a **S**pacer (like a stalk) and a diacyl **L**ipid tail (the anchoring roots). All FSLs are biocompatible and allow users to introduce novel **F**unctional moieties to the membrane surface of living cells[1-3].

Materials & Methods

FSL blood group A (FSL-A), FSL-GB3 and FSL-biotin KODE constructs were supplied by KODE Biotech (www.kodebiotech.com) - figure 1

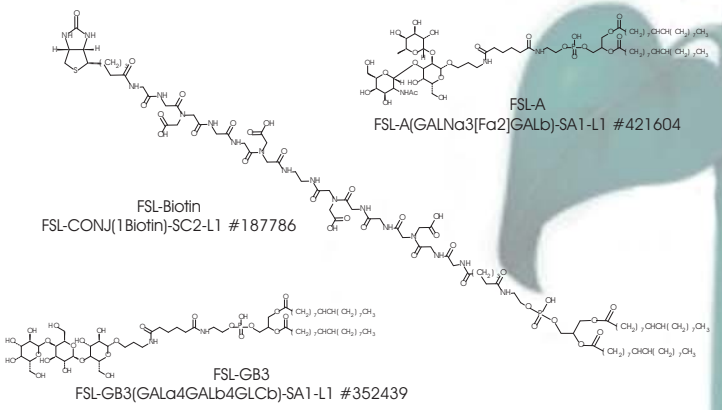

Figure 1. Schematic of FSL-A, the anti-A "incompatible" blood group antigen, FSL-GB3 the "benign" antigen, and FSL-biotin, the visualisation and cell recovery label.

Murine kodecytes bearing both blood group A antigen and biotin (A+biotin kodecytes) were created by incubating equal volumes of packed murine red cells with a solution containing (50μg/ml) FSL-biotin and (100μg/ml) of FSL-A (or FSL-GB3 as negative control). These A+biotin kodecytes or GB3+biotin kodecytes were then transfused (20μl) into the circulation of laboratory mice with or without anti-A (stimulated by immunisation with salivary blood group A substance). Blood was sampled (50μl) at specific time points post transfusion and, using the secondary reagent avidin Alexa Fluor®, surviving kodecytes could be identified in blood films and fluorescence microscopy (figure 2). Additionally by using 50μl of avidin-coated agarose beads (Pierce), in cards transfused biotin kodecytes could be purified from a 5μl whole blood sample (figure 4) for further in vitro analysis after recovery by vortexing from the avidin beads. Anti-A status of all mice was determined using inkjet printed FSL-A constructs in an EIA method[4].

Figure 2. FSL-A was inkjet printed in microwell format. Anti-A status was determined by the appearance of the letter A in the microwell following EIA development[4]

Results

Transfused kodecytes could be identified by fluorescence microscopy (figure 3) in blood films for periods of up to 96 hours in A antigen compatible (series 2) and control (FSL-GB3+biotin and FSL-Biotin alone) mice (series 3-6). When A+biotin-kodecytes were infused into mice with circulating anti-A (series 1) they had significantly reduced (<2hr) red cell survival times (table 1). Using avidin-coated agarose beads transfused A+biotin-kodecytes could be purified from whole blood samples and subjected to further blood group *in vitro* analysis (figure 4).

Table 1. 20μl transfusion of murine kodecytes

Series	Animals (n)	Anti-A	FSL-A+Biotin	FSL-GB3+Biotin	FSL-Biotin	Cell survival (hrs)
1	12	+	+			< 2
2	12	-	+			>96
3	4	+		+		>96
4	1	-		+		>96
5	5	+			+	>96
6	4	-			+	>96

Figure 3. Composite DIC and fluorescence image of series 1 & 2 transfusion

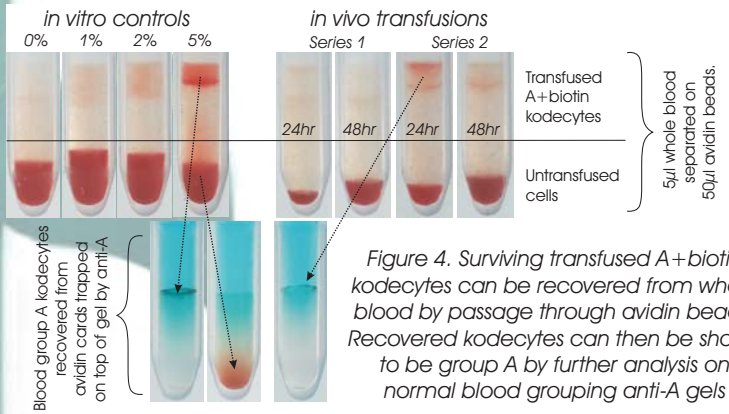

Figure 4. Surviving transfused A+biotin kodecytes can be recovered from whole blood by passage through avidin beads. Recovered kodecytes can then be shown to be group A by further analysis on normal blood grouping anti-A gels

Conclusions

As part of a project investigating ABO incompatible transfusions murine blood group A kodecytes were created with FSL-A. In order to visualise and recover these infused cells they were also co-labled with FSL-biotin. Transfusion survivals were able to be monitored by detecting kodecytes via their FSL-biotin labels and fluorescence microscopy. Surviving transfused kodecytes were also able to be recovered days after transfusion from non-transfused cells by passage through avidin beads which trapped the FSL-biotin kodecytes. These recovered kodecytes could then be analysed in separate *in vitro* tests. The results of this research demonstrate a novel technique for creating deliberate antigen-antibody incompatibility, determining *in vivo* cell survival and recovery of transfused cells for further analysis.

Bibliography

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