Leucocyte depletion filter removes cancer cells in human blood

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Background. Autologous blood transfusion has been avoided in cancer surgery because of the metastatic potential of reinfused tumour cells.

Methods. This study evaluated the efficacy of a blood transfusion filter in removing tumour cells from blood. Whole human blood was admixed with two different malignant cell lines (breast cancer PM1 and MCF7). The blood was filtered through a RC400TE leucocyte depletion filter. Unfiltered blood was used as a control. Detection of malignant cells was performed with immunomagnetic beads and clonogenic assays.

Results. No viable tumour cells were found after filtration with the leucocyte depletion filter.

Conclusion. These findings suggest that the use of a leucocyte filter after intra-operative blood salvage may make autotransfusion safe even in tumour surgery.

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Intraoperative autotransfusion of blood during major surgical procedures is a safe method to reduce the need for homologous blood transfusions (1,2). However, because of the metastatic potential of reinfused tumour cells, its safety in cancer surgery has not been ensured (3, 4).

Earlier reports have demonstrated the possibility of removing tumour cells from blood using blood filters (5, 6). The aim of this study was to evaluate the efficacy of a regular high-flow leucocyte depletion filter in removing cancer cells from human blood.

Material and methods

Cell line. The breast cancer cell line PM1 was established in our hospital from pleural effusion and the MCF7 was obtained from the American Type Culture Collection (Rockville, Maryland, USA). Cells were cultured at 37°C in 5% CO2 in a culture medium consisting of RPMI 1640 medium supplemented with 10% heat inactivated fetal calf serum (FCS).

Blood filter. The blood filter used in these experiments was a RC400TE leucocyte depletion filter (Pall Biomedical Ltd, Portsmouth, UK).

MAbs and immunobeads. The anti breast mucin antibody 2E11 of the subclass IgG3 recognizing an epitope on the muc-1 antigen (7) was a gift from Dr Kaul Heidelberg, Germany. Coating of the primary antibody directly to the a Sheep anti-Mouse IgG Dynabeads M-450 (product no. 427.01., Dynal A/S) was carried out as described by the manufacturers. Briefly, the SAM-Dynabeads were suspended well by vortexing and washed twice in PBS with 0.5% human serum albumin by placing a cobalt samarium magnet to the wall of tubes. After 1 min, the medium was aspirated, and new medium was added to give a final concentration of 15 mg beads/ml. The primary antibody 2E11 was added to the beads in concentration 5ug/mg beads and incubated at room temperature for 2 h during end-over-end rotation. Removal of unbound primary antibody was done by the use of magnets as described by the manufacturers. The prepared beads were stored at 4°C until use.

Experimental conditions. Whole human blood from healthy volunteers, collected in CPD-ADENIN 450 ml bags (Baxter, S.A., La Châtre, France) was admixed with 5×10⁶ breast cancer cell lines PM1 or MCF7. The blood was filtered through a RC400TE leucocyte depletion filter (Pall Biomedical Ltd, Portsmouth, UK). Unfiltered blood containing the same amount of tumour cells were used as a control. The 450 ml blood
samples were centrifuged and concentrated to a final volume of 40 ml in 50 ml tubes. The desired number of 2E11 dynabeads was added to the tubes and incubated at 4°C for 30 min under gentle rotation. The optimal ratio of beads to antibody-binding cells was found to be 50 to 1 (data not shown), and this ratio was used in all experiments.

In all cases, the resulting cell/bead aggregates were removed by placing a cobalt samarium magnet to the wall of the tubes. After 3 min, the supernatant was removed and the bead/cell complexes were resuspended in RPMI medium containing 10% fetal calf serum and seeded out in 50 ml tissue culture flasks (Nunclon, Nunc A/S, Roskilde, Denmark). The tissue flasks containing the filtered blood were inspected daily up to 1 week with microscopy. When a confluent layer of tumour cells was observed by microscopy in the corresponding control tissue flasks after an examination period up to 1 week, the cells were trypsinized and the number of viable cells was calculated.

Results
No growth of malignant cells was detected in the tissue culture flasks containing the filtered blood (Table 1), examined up to 1 week after seeding, while numerous tumour cells were observed in the tissue culture flasks containing unfiltered blood (Fig. 1). The number of cells with clonogenic capacity increased gradually until a layer of tumour cells was developed and calculation of the viable cells was performed as shown in Table 1.

Discussion
In this study, no clonogenic tumour cells were found after filtration with a leucocyte depletion filter, evaluated by microscopy and clonogenic assays after selection with immunomagnetic beads.

There are several reasons for restricting the use of homologous blood. One of the methods for reducing the need for banked blood is autotransfusion using a cell saver. However, autotransfusion of intra-operative salvaged blood is traditionally contraindicated in cancer surgery due to the concern that tumour cells from the operating field may be reinfused into the patient (3, 4). One study demonstrated that isotopically labelled tumour cells were detected mainly within the red cell concentrate after processing with a cell saver (4). Furthermore, it has been shown that centrifugation and washing of the blood decrease, but do not eliminate, the tumour cells (4, 8).

Filtration of processed blood, before retransfusion, using a RC400TE leucocyte depletion filter may be a reasonable option since tumour cells were completely removed in this study. While earlier leucocyte depletion filters limit the rate of transfusion and the use of pressure infusors, the Pall RC400 is a rapid flow filter also compatible with the use of a pressure cuff.

Using the immunomagnetic selection technique to enrich the blood to be investigated with tumour cells, allowed us to employ a highly sensitive method to detect the cancer cells. Previous experiments (data not shown) have demonstrated that the method can recover one cancer cell in $1 \times 10^6$ cells. Clonogenic assays, furthermore, demonstrated that cancer cells, possibly not detected by microscopy, did not have any in vitro clonogenic capacity.

However, further studies should be performed before autotransfusion of salvaged blood with a cell saver in addition to a leucocyte depletion filter should become routine practice in cancer surgery.