

# Continuous, Flow-Through Immunomagnetic Cell Sorting in a Quadrupole Field

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Received 17 October 1997; Revision Received 21 May 1998; Accepted 28 July 1998

A flow-through quadrupole magnetic cell separator has been designed, built, and evaluated by using a cell model system of human peripheral T lymphocytes (CD4<sup>+</sup>, CD8<sup>+</sup>, and CD45<sup>+</sup> cells). The immunomagnetic labeling was accomplished by using a sandwich of mouse anti-human monoclonal antibody conjugated to fluorescein isothiocyanate and rat anti-mouse polyclonal antibody conjugated to a colloidal magnetic nanoparticle. The feed and sorted fractions were analyzed by FACScan flow cytometry. The magnetically labeled cells were separated from nonlabeled ones in a flow-through cylindrical column within a quadrupole field, which exerted a radial, outward force on the magnetic cells. The flow rate of the cell samples was 0.1–0.75 ml/min, and the flow rate of sheath fluid was 1.5–33.3 times that of the sample flow rate. The maximum shear stress exerted on the cell was less than 1 dyne/cm<sup>2</sup>, which

was well below the level that would threaten cell integrity and membrane disruption. The maximum magnetic field was 0.765 T at the channel wall, and the gradient was 0.174 T/mm. The highest purity of selected cells was 99.6% (CD8 cells, initial purity of 26%), and the highest recovery of selected cells was 79% (CD4 cells, initial purity of 20%). The maximum throughput of the quadrupole magnetic cell separator was 7,040 cells/s (CD45 cells, initial purity of 5%). Theoretical calculations showed that the throughput can be increased to 10<sup>6</sup> cells/s by a scale-up of the current prototype. *Cytometry* 33: 469–475, 1998.

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**Key terms:** quadrupole field; immunomagnetic label; flow cytometry; FACS; flow cell sorting; magnetic cell separation

The capability to separate a heterogeneous cell population based on the expression of a surface marker has become a significant preparative technology (17). The applications of this technology range from basic biological studies to clinical practice (18). To achieve these separations, a number of techniques based on different types of antibody-linked interactions are being used. These different techniques include fluorescent labels conjugated to antibodies (immunofluorescent labels), support matrices conjugated to antibodies (immunoaffinity column or “panning”), and magnetic labels conjugated to antibodies (immunomagnetic labels).

Of these different separation approaches, probably the most versatile and the best characterized is an automated immunofluorescence sorting technique, fluorescence activated cell sorting (FACS). FACS is widely used for both cell analysis and sorting and is capable of sorting with high purity and recovery of cells. However, its high cost and relatively low throughput (on the order of 10<sup>4</sup> cells/s) are significant limitations (17). Because of these limitations, less expensive but cruder bulk cell separation methods have been developed. An example of an immunoaffinity

technique used for bulk cell separation is an immunoaffinity column, CEPRATE, developed by CellPro (Bothell, WA) (1). This system, by its very nature, is based on a batch process, but high throughput (10<sup>6</sup> cells/s) can be obtained. However, compared with FACS, its purity and recovery are lower. Several commercial and research devices, representative of the bulk immunomagnetic cell separation technique, are also available. These immunomagnetic techniques can be classified based on the size of the magnetic label, ranging from particles on the order of a cell diameter (1–5 μm) to colloids (0.05–0.5 μm iron-dextran) to molecules (≈12 nm, ferritin) (12,13,19,20). The performance of immunomagnetic systems is governed

This paper was presented in part as a poster at the 26th Annual Meeting of the International Society for Experimental Hematology, Cannes, France, 24–28 August 1997.

Grant sponsors: NIH; Grant numbers: CA62349 and NSF BCS 9258004; Grant sponsor: Whitaker Foundation.

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by the size of the label and mode of operation, which will be discussed further below.

The driving force in immunomagnetic cell separation is the force due to the magnetic field,  $F_m$ , acting on the cell through the combination of immunomagnetic labels and a magnetic field gradient. This force is represented by the following equation (in SI units):

$$F_m = \Delta\chi V \text{grad} \left( \frac{B^2}{2\mu_0} \right) \quad (1)$$

where  $\Delta\chi$  is the volumetric magnetic susceptibility of the labeled cells relative to medium,  $V$  is the volume of one cell,  $\text{grad}(B^2/2\mu_0)$  is the magnetic field energy gradient, and  $\mu_0$  is the magnetic permeability of free space. Because, in general, the magnetic force imparted on an immunomagnetically labeled cell is directly proportional to the volume, number, and magnetic susceptibility of the magnetic labels, the type of label used has a large impact on the design of the separation device.

If a high magnetic field gradient could be achieved, it would be possible to design a flow-through separator that could combine some of the advantages of the FACS system with those of magnetic separations to achieve high purity, high recovery, and high throughput separation. In addition, if a direct proportionality existed between the magnetic label and the cell surface marker, it would then be possible to separate cells based on cell surface marker density.

The remainder of the present study concerns the design and testing of a high magnetic field gradient, flow-through, separation system that can separate cells labeled with colloidal and molecular labels. The separator was evaluated with a cell model system of human peripheral T lymphocytes ( $CD4^+$ ,  $CD8^+$ , and  $CD45^+$  cells).

## MATERIALS AND METHODS

### Mononuclear Cells From Peripheral Blood

Human peripheral blood was obtained from apparently healthy volunteer donors in accordance with the institutional guidelines. The Ficoll-Paque technique was used to obtain peripheral blood mononuclear (PBMN) cells, which were isolated by layering diluted blood over a Ficoll cushion (Pharmacia Biotech, Uppsala, Sweden) and centrifuging and collecting cells at the Ficoll interface.

### Immunomagnetic Labeling of Lymphocytes

A previously published, two-step immunofluoromagnetic labeling procedure was employed (21). This procedure allows for both magnetic separation and flow cytometry analysis. The cells were first labeled with mouse anti-human CDx monoclonal antibodies (mAb) conjugated to fluorescein isothiocyanate (FITC; Becton-Dickinson Immunocytometry Systems, San Jose, CA). Next, the cells were labeled with rat anti-mouse polyclonal antibodies (pAb) conjugated to iron dextran colloid (MACS microbeads, Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). In addition to the test sample preparation, a

control was prepared with IgG-FITC isotype from which the threshold gate was set for the positive cell determination during FACS analysis. Cells were washed after each labeling step to remove unbound reagents from solution and were resuspended in medium comprising phosphate buffered saline (PBS) with 1.1% bovine serum albumin (BSA; Ortho Diagnostics Systems Inc., Raritan, NJ) and 0.05% sodium azide.

### Quadrupole Magnetic Field and Sorter Design

Our magnetic separator is based on a quadrupole magnet design that has been used for many years in quadrupole mass spectrometry and has been tested for continuous separation of coal fractions (4,5,9). The use of a quadrupole field for flow cell separation has been suggested, but no results have been published showing cell separation in continuously flowing viscous medium (9). A quadrupole magnetic field was chosen because of several unique characteristics. Some of these characteristics are linearly increasing (in the radial  $r$  coordinate) field intensity (independent of angular,  $\theta$ , and axial,  $z$ , coordinates), and a constant, high magnetic field gradient along the  $r$  coordinate. Mathematically, the magnetic force imparted on an immunomagnetically labeled cell in the quadrupole field is represented by

$$F_m = \Delta\chi V \left( \frac{B_0^2}{\mu_0 r_0} \right) \frac{r}{r_0} \quad (2)$$

where  $B_0$  is the magnetic flux density at the pole tips, and  $r_0$  is the location of the tip of a pole (22).

In the quadrupole magnet, four magnetic poles focus the magnetic field around a central, cylindrical area. Two cylindrical flow splitters, an outer cylinder and a coaxial solid rod, constitute the flow device. A perspective drawing (Fig. 1A) and side (Fig. 1B) and top (Fig. 1C) views are shown in Figure 1. The separator flow channel was contained between two coaxial brass cylinders of diameters 4.76 mm and 8.82 mm. To prevent oxidation and provide smooth flow surfaces, the surfaces that contacted the cell suspension were polished and gold plated. Cell suspensions and sheath fluid were delivered to and collected from the separator through the use of syringe pumps (Harvard Apparatus, Inc., South Natick, MA).

### Magnet Specifications

The quadrupole magnet was made from four pairs of permanent magnets ( $2 \times 2 \times 0.5$  inches; neodymium-iron-boron, Dexter Magnetics Corp., Toledo, OH) characterized by a maximum energy product of  $2.23 \times 10^5$  T-A/m, and four steel pole pieces with circular shape at the pole tips (Fig. 1). The magnetic field was measured by a Gaussmeter and Hall-effect probe (Model 9200 Gaussmeter and transverse probe STG920404, F.W. Bell, Orlando, FL). A precision translation stage (Model 420 X-Y Movement, Newport Research Corp., Fountain Valley, CA) was used to facilitate precise positioning of the probe in the magnetic field. The field was mapped over approximately

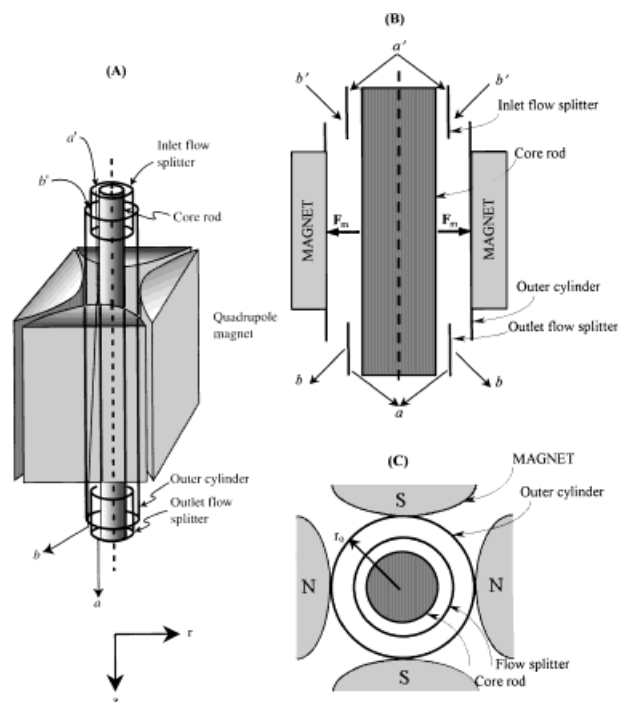


FIG. 1. A perspective (A), side (B) and top (C) views of the quadrupole magnetic flow sorter. The magnetically-labeled cells experience radial, outward force of the quadrupole magnetic field, cross the flow streamlines, and are collected at outlet part *b*. The non-labeled cells follow the flow streamlines, and are collected at outlet part *a*.

50% of the aperture cross-sectional area at a spatial resolution of 0.32 mm. Linear regression of  $B$  on  $r$  was obtained with a correlation coefficient of 0.989 ( $n = 60$ ). The slope and constant parameter of the regression remained independent of the angular coordinate (constant to within 1.7%), in agreement with equation 2. The field flux density  $B_0$  measured at the pole tips was 0.829 T, and a constant magnetic gradient in the radial direction,  $B_0/r_0 = 0.174$  T/mm, was achieved. The field measurement results were in agreement with the theoretical expressions for the quadrupole field (equation 2). The magnetic flux density increased linearly with the radial coordinate,  $r$ , as predicted by equation 2.

### Cell Separation Process

A cell suspension, consisting of paramagnetically labeled cells, is introduced into the inner, inlet flow (between the core rod and splitter; labeled *a'* in Fig.1), and the sheath fluid is introduced into the outer, inlet flow (between the splitter and outer cylinder; labeled *b'*). Within the region of focused magnetic field, the splitter is omitted, which allows labeled cells to move in an outward radial direction into the sheath fluid flow as a result of the magnetic force acting on the cell as indicated by equation 2. The unlabeled or weakly labeled cells do not move sufficiently in the radial direction to be separated from the labeled cells at the outlet fluid flow streamlines. Once the fluid, containing labeled and unlabeled cells, flows to the end of the magnetic field domain, the cylindrical outlet

flow splitter separating the two flows reappears and the fluid is collected in an outer, outlet flow, labeled *b*, and an inner, outlet flow, labeled *a*. The separated flow streamlines carry the magnetically separated cells to different outlets—the nonmagnetic ones to outlet *a* and the magnetic ones to outlet *b*.

### Separator Operation and Evaluation

The separator was primed with degassed sheath fluid (calcium- and magnesium-free PBS with 1.1% BSA, 0.05% sodium azide), from bottom to top. The separation was implemented by starting all three pumps (for streams *a'*, *b'* and *b*) at the same time. The fourth stream *a* was not attached to a pump to allow for pressure equilibration within the system. When the test sample (feed) syringe was almost empty, the three pumps were stopped at the same time. The volumes of collected fluids were determined by weight, and cell concentrations were determined with a Z-1 Coulter Counter (Coulter Corporation, Miami, FL). The fractional cell content (purity) in each sample was determined by FACS.

The selection of proper ratio of the sample feed to sheath flow rates,  $\dot{V}_a:\dot{V}_b$  and exit ratio,  $\dot{V}_a:\dot{V}_b$ , is critical to successful operation of the magnetic flow sorter. In particular, this ratio affects purity of the magnetically-labeled cells in fraction *b*. Less than ideal (100%) purity of the target cells in fraction *b* is the result of cell cross-over to fraction *b* due to effects other than magnetic, such as channel imperfections and flow mixing. In preliminary studies, we tested cell cross-over by using 11.9- $\mu$ m polymeric, nonmagnetic beads (Seradyn, Indianapolis, IN) for a series of different values of  $\dot{V}_a:\dot{V}_b$ , from 1:19 to 1:4 at a constant  $\dot{V}_a:\dot{V}_b$ . We found that the bead cross-over decreases with decreasing  $\dot{V}_a:\dot{V}_b$ , as expected, and that it produces a satisfactory, low bead recovery of 1% to 3% in fraction *b* at  $\dot{V}_a:\dot{V}_b = 1:19$  to 1:9 (unpublished data). We choose those low values of  $\dot{V}_a:\dot{V}_b$  in the experiments with lymphocytes (Table 1). In a series of preliminary experiments with the immunomagnetically labeled lymphocytes, we observed increasing purity and a concomitant decrease in recovery of the target cells in fraction *b*, with the increasing  $\dot{V}_a:\dot{V}_b$  at a constant  $\dot{V}_a:\dot{V}_b$ . We chose  $\dot{V}_a:\dot{V}_b = 1:4$  to 1:2 in experiments presented in this work (Table 1).

### Flow Cytometry Analysis

All the cell samples collected for flow cytometry analysis were washed, fixed in 0.5% paraformaldehyde solution, and stored overnight in the refrigerator. Data acquisition and analysis of the cell samples were performed using FACScan flow cytometer and Cell Quest software (Becton Dickinson).

### RESULTS

Three different cell surface markers were used to demonstrate feasibility of the flow-through quadrupole magnetic cell sorting. The sorting results were evaluated in the context of cell enrichment, depletion, or both. The control over enrichment or depletion could be accom-

Table 1  
Separation Results of CD4<sup>+</sup>, CD8<sup>+</sup>, and CD45<sup>+</sup> Lymphocytes\*

Target cell	CD4 <sup>+</sup> cell	CD8 <sup>+</sup> cell		Non-CD45 <sup>a</sup> cell	Rare CD45 <sup>+b</sup> cell		
No. experiments <sup>c</sup>	1	2		1	3		
Purity (%)							
<i>a'</i>	20	15	26	6	3	3	5
Total cell concentration in feed × 10 <sup>-6</sup> (cells/ml)							
<i>a'</i>	1.26	0.88	0.932	0.690	2.28	2.28	3.52
Target cell concentration in feed × 10 <sup>-6</sup> (cells/ml)							
<i>a'</i>	0.256	0.132	0.242	0.041	0.068	0.068	0.176
Flow rate (ml/min)							
<i>a'</i>	0.2	0.3	0.3	0.25	0.12	0.12	0.12
<i>b'</i>	3.8	2.7	2.7	4.75	3.88	3.88	3.88
<i>a</i>	0.8	0.9	0.9	1.0	0.8	0.8	0.8
<i>b</i>	3.2	2.1	2.1	4.0	3.2	3.2	3.2
Purity (%)							
<i>a</i>	8	9	12	37	1	1	3.7
<i>b</i>	97	96	99	0	85	77	79
Recovery (%)							
<i>a</i>	14.8	38.9	25.4	77.9	6.2	9.7	11.4
<i>b</i>	79.1	58.3	64.4	10.6	68.0	68.3	77.6
Enrichment							
<i>a</i>	0.34	0.57	0.38	9.24	0.24	0.33	0.85
<i>b</i>	121.9	137.4	147.1	0.14	114.3	76.0	81.8
Total cell concentration in sorted fractions × 10 <sup>-3</sup> (cells/ml)							
<i>a</i>	54.4	154	187	7.89	53.7	38.0	33.4
<i>b</i>	8.98	9.72	7.30	13.1	1.71	1.34	2.89
Target cell concentration in sorted fractions × 10 <sup>-3</sup> (cells/ml)							
<i>a</i>	4.35	13.86	22.44	2.92	0.54	0.38	1.24
<i>b</i>	8.71	9.33	7.23	0.05	1.45	1.03	2.28
Throughput (cells/s)	4200	4400	4660	2880	4560	456	7040

\*Each experiment was reported because of the variation in purity in feed and the dependence of sorting results on purity in feed.

<sup>a</sup>The entire sample was stained with αCD45 monoclonal antibodies.

<sup>b</sup>Unstained human lymphocytes were mixed with small fraction of αCD45 monoclonal antibody-stained cells.

<sup>c</sup>The experiments were reported for the flow rates as shown. In a previous study, we evaluated the effects of the changing flow conditions, which are not shown here.

plished by choosing different operating parameters (flow rates in channels *a'*, *b'*, *a*, and *b*) and analyzed by process simulation, which is not discussed in the present study.

The cell sorting process took place in the continuously flowing liquid medium. We observed a small fraction of target cells collected on the walls of the flow channel, which could have been related to their higher-than-average magnetization. The effect of cell magnetization distribution on the magnetic flow sorting process has been discussed elsewhere (3). The cells adhering to the walls were washed off the channel after the flow sorting experiment, outside the magnet, and analyzed by flow cytometry and counted. The target cell fraction collected on the wall varied from 5% (CD4 cells) to 17% (CD8 cells). It is expected that the fraction of cells collected on the channel walls will become less significant in future experiments with large cell volumes. Because of limited feed volume, the volume of the cell sample, 3 ml, was less than the volume of the flow channel, 7 ml, in current experiments, which exacerbated cell losses due to wall adhesion.

Both enrichment and depletion were achieved with one pass through the system with paramagnetically labeled CD4 human lymphocytes (Fig. 2). The upper part presents

a FACS histogram of a cell suspension before being introduced into the separator (fraction *a'*). As indicated, 20% of the cells are labeled positive for CD4. To assist in the interpretation of these data, a schematic cross-section of half of the separator is shown in the middle part of Figure 2 (compare also Fig. 1B). Lower parts present the histograms of the outlet flows. Ninety-seven percent of the cells in the outer outlet flow *b* are labeled CD4 lymphocytes, whereas 8% of the cells in the inner outlet flow *a* are positively labeled. In this case, approximately 4,200 cells were processed per second, and a total of 3,780,000 cells were sorted per run.

A second experiment (Fig. 3) demonstrated the potential of cell enrichment of CD8 labeled human lymphocytes. In this case, 15% of the feed was positively labeled; the outer outlet flow *b* was enriched to 96% CD8 cells, and the inner outlet flow *a* was depleted to 9%. Approximately 4,400 cells were processed per second, and a total of 5,280,000 cells were sorted per run.

In some experiments, we used a cell model system of unlabeled lymphocytes spiked with a controlled amount of lymphocytes labeled with anti-CD45 antibody to assess the capability of the system for rare cell depletion and

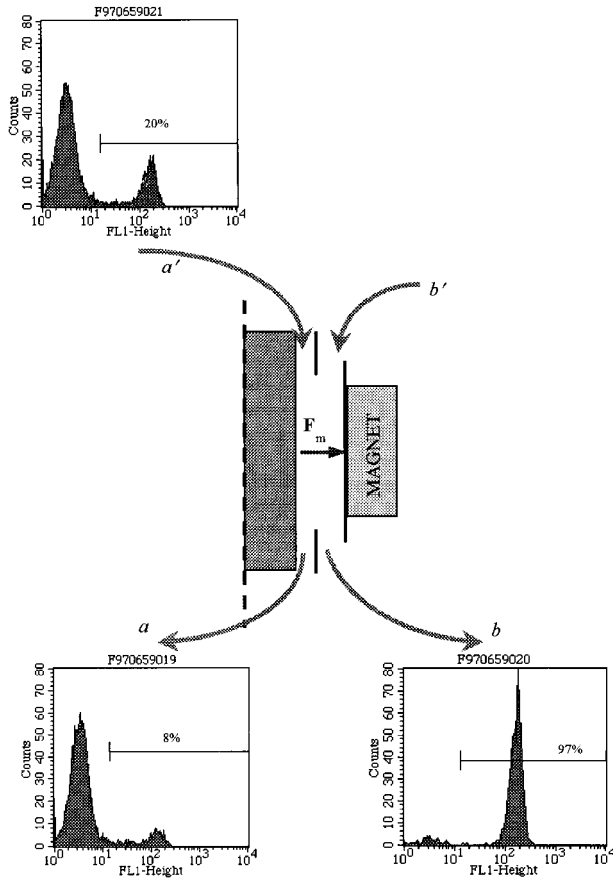


FIG. 2. Positive cell depletion by magnetic flow sorter (single pass). CD4 cell immunofluorescence histograms of the feed cell suspension (a') and the two sorted fractions (a and b). See Table 1 for details.

enrichment. The CD45 marker is expressed on all lymphocytes; therefore, such experiments are of little direct practical value. Indirectly, such experiments provide valuable information about expected performance of the system for such applications as residual tumor cell depletion or rare hematopoietic stem cell enrichment.

A third experiment (Fig. 4) demonstrated the potential of depletion of unlabeled cells from CD45 labeled human lymphocytes. In this case, 6% of the feed (a') contained CD45-negative cells; the outer outlet flow b was depleted to 0.4% CD45-negative cells, and the inner outlet flow a contained 37% of CD45-negative cells. Approximately 4,000 cells were processed per second, and a total of 3,000,000 cells were sorted per run. This experiment indicated the expected performance of the sorter when applied to the depletion of unlabeled cells, such as tumor cells from an autograft.

To test the potential of the method to enrich rare cells, such as hematopoietic stem cells, samples of labeled CD45 cells were mixed with unlabeled human lymphocytes (Fig. 5). In this case, 3% of the feed a' was CD45 positive; the outer outlet flow b was enriched to 85% labeled CD45 cells and the inner outlet flow a was depleted to 1%.

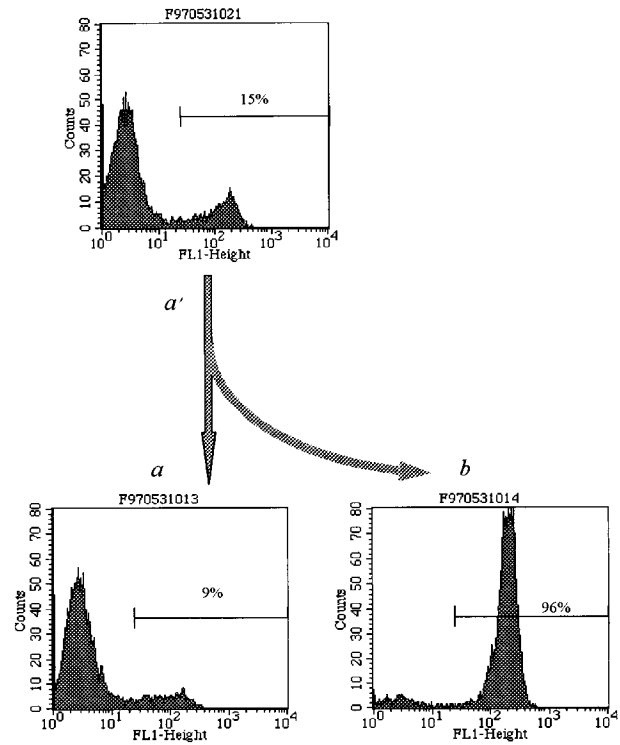


FIG. 3. Positive cell enrichment by magnetic flow sorter (single pass). CD8 cell immunofluorescence histograms of the feed suspension (a') and the two sorted fractions (a and b).

Approximately 6,000 cells were processed per second, and a total of 5,400,000 cells were sorted per run.

The results of the above experiments are summarized in Table 1. The recovery and enrichment rate are calculated with the following relationships:

$$R_i = \frac{n_{CDx,i}}{n_{CDx,f}} \quad (3)$$

$$E_i = \frac{P_{CDx,i}/P_{CDx,f}}{(1 - P_{CDx,i})/(1 - P_{CDx,f})} \quad (4)$$

where x = 4, 8, and 45 were used to indicate different cell surface markers, and subscript i = a or b indicated outlet fractions, respectively. Subscript f indicated the feed sample. R and E denoted cell recovery and enrichment rate, respectively. The letters n and P express cell number and purity, respectively. Purity is the fractional concentration of the cells of interest as measured by FACS.

### DISCUSSION

Two of three commercial immunoseparation approaches mentioned in the present study operate in a batch mode (except for FACS). Although batch processes have the advantages of relatively simple separator design and high depletion rates (adsorption and removal of undesirable

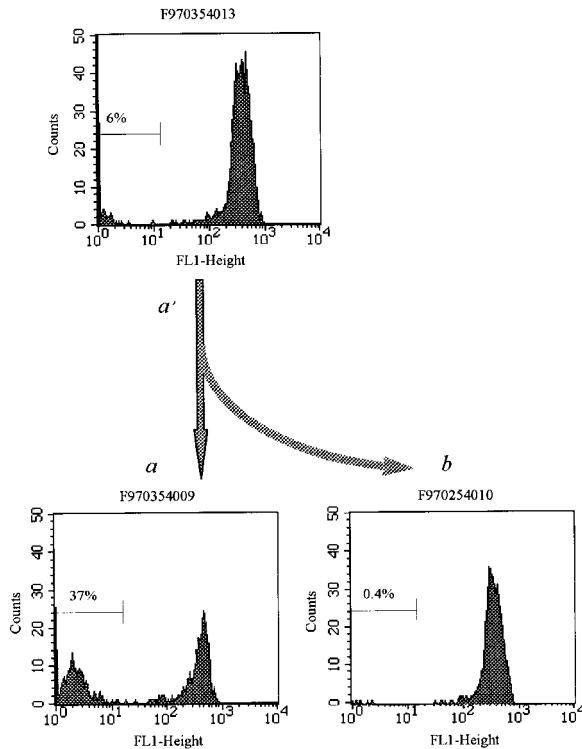


FIG. 4. Negative cell depletion by magnetic flow sorter (single pass). CD45 cell immunofluorescence histograms of the feed suspension (a') and the two sorted fractions (a and b).

cells), they are intrinsically limited in their throughputs and enrichment rates (adsorption and recovery of desirable cells). Continuous magnetic cell sorting has the potential to overcome these limitations by sorting cells in a flowing solution without cell physical contact with solid walls.

There are three different types of magnetic labels based on size. The use of immunomagnetic particles leads to a large difference (up to five orders of magnitude) in the magnetic susceptibility between the unlabeled cells and the cell-magnetic particle complex. This large difference in principle could lead to high-resolution separation of labeled and unlabeled cells. In a typical cell depletion application, by using a Dynabead-based separation system (MPC separator series, Dynal AS, Trondheim, Norway), high depletion rates (98%) were achieved in purging bone marrow of CD8 cells with concomitant moderate losses of CD4, CD20, and CD34 cells (15%) (7). However, using particulate magnetic labels decreased the resolution by a number of unwanted effects leading to paradoxical results (10,15). Some of the causes of these unwanted effects include nonspecific binding of beads to nontarget cells, random entrapment of unlabeled cells in bead clusters, and detachment of beads from target cells (sometimes with the cell membrane fragments) because of high shear stresses created by rapidly accelerating magnetic beads (15).

Unlike the particulate labels, colloidal and molecular magnetic labels require much higher magnetic field gradi-

ents (because of the much smaller label size) to achieve sufficiently high magnetic force to affect cell movement in a fluid. With current commercial separation units that use colloidal labels, high magnetic field gradients are achieved through the use of small columns filled with high magnetic permeability matrices (14). The overall performance of one such system, the MACS unit (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) is comparable to that of the Dynabead-based separation system, with the Dynabead-based system being more efficient for cell depletion and the MACS system being more efficient for cell enrichment (11). High purity and recovery of the positive cell selection become important to enrich rare cells for analytical and preparative purposes, such as the identification of fetal cells in the maternal blood and isolation of progenitor and stem cells from bone marrow (2,16). High depletion purging is also essential for clinical applications, for example, purging bone marrow of cytotoxic T lymphocytes and tumor cells (6). Another difference between particulate, colloidal, and molecular magnetic labels is that the smaller immunomagnetic labels (colloidal and molecular) have the distinct potential advantage of imparting a magnetic force proportional to the number of surface markers on the cell (3).

The feasibility of a continuous immunomagnetic cell sorting with colloidal magnetic labels have been demonstrated in this study. Throughput of the cells through the system was kept low ( $1 \times 10^3$  to  $2 \times 10^4$  cells/s) because of the scarcity of peripheral blood cells available for the

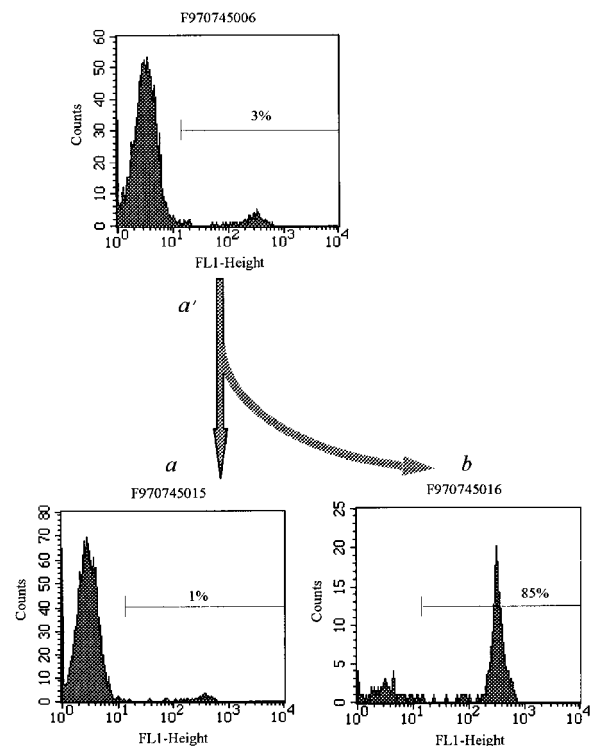


FIG. 5. Rare positive cell enrichment by magnetic flow sorter (single pass). Small number of magnetically-labeled CD45 cells was mixed with unlabeled lymphocytes, fraction a', and sorted into fractions a and b.

present study and the resulting low concentration of cells in the feed sample.

In the course of the present study, a number of interesting trends were noted that were not pursued further because of the preliminary character of this work. In particular, we observed that the geometrical mean cell immunofluorescence intensity is higher in the enriched fraction than in the original and the depleted cell fractions. This finding may be noted by inspection of Figure 3, which shows separation results for CD8 cells. Such trends suggest the possibility of using precisely controlled flow conditions to sort cells differing in surface antigen number. The preliminary studies of this effect have been reported elsewhere (8). The implications for the magnetic flow sorting have been analyzed at length in previous studies (3).

We reported previously that a significant analogy can be drawn between the split-flow lateral transport thin-channel separation (SPLITT) system and the hydrodynamic properties of flow in the cylindrical columns in the quadrupole separator (23). Another analogy can be drawn between the interactions of the quadrupole magnetic field acting on the dipole moment of the magnetic labels and centrifugal field acting on a mass. Both forces are proportional to the radial distance from the axis of symmetry (equation 2). Both the theoretical and experimental implications of these analogies are currently being investigated. Preliminary results indicate that it is possible to control the purity, recovery, and the cell surface marker density of the sorted cells by controlling the flow parameters of the quadrupole sorter. We are currently developing mathematical and experimental models to simulate this separation process. Theoretical estimates of larger designs, higher cell concentrations, and higher flow rates through the system indicate that a 100-fold increase in cell throughput is realistic.

#### ACKNOWLEDGMENTS

We thank Jim Proudfit for his expert machining of flow channel and magnetic pole pieces and Amy Raber for running the FACS analysis. This study was supported by NIH grants CA62349 (M.Z.) and NSF BCS 9258004 and by Whitaker Foundation grants (J.J.C.).

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