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**Searching for Common Stem Cells of the Hepatic and Hematopoietic Systems in the Human Fetal Liver: CD34<sup>+</sup> Cytokeratin 7/8<sup>+</sup> cells Express Markers for Stellate Cells**

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## **Abstract**

The hematopoietic and hepatic systems are intertwined in the liver during fetal life. Cells expressing the hematopoietic stem cell marker CD34 and cytokeratin 7/8 (CK7/8) are hypothesized to be common stem cells for the hematopoietic and hepatic systems. Aim: To determine if human fetal liver cells expressing CD34 and CK7/8 represent a common stem cell for both the hematopoietic and hepatic systems. Method: CD34<sup>+</sup>CK7/8<sup>+</sup> cells from midgestation livers were analyzed for the expression of various markers by flow cytometry and isolated based on their expression of CD34, nerve growth factor receptor (NGFR) and lack of CD45 expression. CD34<sup>+</sup>CD38<sup>-</sup> hematopoietic stem cells were also isolated and cultured in the presence of various hepatopoietins. Results: CD34<sup>+</sup>CK7/8<sup>+</sup> cells comprised 3.4%-8.5% of the erythrocyte-depleted liver. CD34<sup>+</sup>CK7/8<sup>+</sup> cells had unique light-scatter properties compared to hematopoietic precursors and did not express most markers associated with hematopoietic cells. They did stain with CD13, CD59, NGFR, desmin and  $\alpha$ -smooth muscle actin. In culture, these cells had a stellate appearance. Cultured hematopoietic stem cells failed to generate hepatocytes. Conclusion: CD34<sup>+</sup>CK7/8<sup>+</sup> cells are not common stem cells but rather appear to be hepatic stellate cells. A link between the hematopoietic and hepatic systems during fetal life requires further investigation.

## **Introduction**

During human fetal development, the hepatic and hematopoietic systems are intertwined. The liver forms from an endodermal outgrowth of primitive foregut around the third to fourth week gestation (1). Hepatoblasts, the pluripotent cells of the outgrowth, give rise to both hepatic and biliary cells. They form a double-layered cylinder of cells called the ductal plate. The ductal plate cells migrate into surrounding mesenchyme to form intrahepatic bile ducts. Those cells not in contact with the portal mesenchyme differentiate into hepatocytes (1). During the fifth week of human gestation hematopoiesis moves from the yolk sac and/or adreno-gonadal-mesonephric (AGM) to the liver. The liver is the principal hematopoietic organ in the human fetus from the 6<sup>th</sup> week through mid-gestation (2). During this time, hematopoietic stem cells can be isolated from human fetal liver. In the adult liver, hematopoiesis can re-emerge during times of extreme stress. It is because of this intertwining of the hepatic and hematopoietic systems that a common stem cell has been proposed.

In both animal and human studies, multipotent hepatic stem cells have been shown to exist within the liver. Both ductal plate cells, during fetal development, and the hepatic oval cells, the presumed hepatic progenitor cells seen during liver regeneration, are at least bipotential. They are able to differentiate into both hepatocytes and biliary cells. Oval cells, observed during liver regeneration, proliferate when liver damage is severe with widespread hepatocyte necrosis or when hepatocyte proliferation is prevented by exposure to hepatotoxins. The oval cells appear initially in the periportal region around the biliary ductules of liver lobules, and then appear to migrate into the hepatic parenchyma. They are described as small and ovoid, with scant cytoplasm. Both ductal plate cells and oval cells express CD34, which is also expressed on hematopoietic stem cells (3-6). Activated oval cells also express CD117 (c-kit) and

CD90 (Thy-1), markers commonly found on hematopoietic stem cells (7). In addition, ductal plate and oval cells express cytokeratins 7 and 8 (CK7/8), which can be detected using the monoclonal antibody (mAb) CAM5.2. Van Eyken et al. showed that ductal plate cells stain strongly with CAM5.2 while primitive hepatocytes within the developing fetus stain weakly (8). CK7/8 are not found in hematopoietic cells (9).

Despite the liver's clear role as a hematopoietic organ during fetal life, the developmental relationship between hematopoietic stem cells and the hepatic system remains unclear. Hematopoietic stem cells have been shown to differentiate into hepatic and biliary cells in the adult liver. A number of animal studies have shown a connection between the hematopoietic system and hepatic system. Petersen et al. irradiated female rats and transplanted them with allogenic bone marrow cells. A proportion of regenerated cells was shown to be donor derived based on the presence of donor-cell markers such as Y chromosome, dipeptidase IV enzyme, and L21-6 antigen (10). In a study by Lagasse, et al., intravenous injection of adult bone marrow cells in the FAH<sup>-/-</sup> mouse, an animal model of tyrosinemia type 1, rescued the mouse and restored the biochemical function of the liver (11). In humans, repopulation of the liver with male donor cells was seen in female bone marrow transplant patients who received male donor hematopoietic cells. In addition, male patients undergoing liver transplantation with a female liver had evidence of male cells within the liver (12). These findings indicate that colonization of the hepatic system can occur from other tissues, possibly from cells of hematopoietic origin. Recently, bone marrow cells have been shown to fuse with cells of other tissues (13-15). In the liver, hematopoietic cells after cell fusion adopt a more hepatocyte-specific profile and lose hematopoietic markers (16). The development of these "transdifferentiated" cells is believed by some to represent the pathway for which the hematopoietic and hepatic systems intertwine. If the

hepatic, biliary and hematopoietic cells do share a common stem cell, then the fetal liver is likely to contain these cells. Intermediary progenitors for the hepatic, biliary and hematopoietic lineages should also be enriched in the fetal liver since these lineages are all actively growing in the fetus. In previous studies, we have phenotypically characterized the hematopoietic stem cell compartment within the human fetal liver. Fetal hematopoietic stem cells reside within a population of cells that express the cell surface markers CD4, CD34, CD90 and CD117, but lack expression of CD38 (17, 18). There have been few studies characterizing hepatic and biliary progenitors in the fetal liver. Recently, a population of fetal liver cells that co-expressed CD34 and CK7/8 was proposed as a candidate common-stem cell of the hepatic and hematopoietic lineages (6). Our aim in this study was to further characterize this presumed common-stem cell population and to assign its position in the hierarchy of hematopoietic, hepatic and/or biliary cells.

## Materials and Methods

### *Isolation of fetal liver cells*

Livers were harvested from mid-gestation fetuses after elective abortions and were obtained with maternal consent. Gestational ages were estimated based on foot length and ranged from 17 to 23 weeks for the tissue analyzed in this study. Research was performed with approval of the Committee of Human Research at our institution.

The CD34<sup>+</sup>CD38<sup>-</sup> population of fetal liver cells, known to be enriched for hematopoietic stem cells (17, 18), was isolated as previously described in detail (19). For phenotypic analysis, fetal liver cells were isolated by a modification of the previously described protocol. Livers were physically dissociated and CD235a<sup>+</sup> erythrocytes were depleted using immunomagnetic beads, thereby enriching the remaining cell types that comprise the fetal liver. Additionally, the previously described procedure was modified by reducing the *g*-force used during centrifugation steps to 50x *g*, to better maintain the viability of delicate hepatic cells. Furthermore, the density fractionation and second immunomagnetic-bead depletion steps were omitted. CD34<sup>+</sup>NGFR<sup>+</sup>CD45<sup>-</sup> cells were isolated by flow cytometry using the above modified-technique in conjunction with surface staining with CD45-fluorescein isothiocyanate (FITC), NGFR-phycoerythrin (PE) and CD34-PE-cyanine 5 (PC5).

### *Flow cytometric analysis of cell surface and intracellular antigens*

Cells were suspended in 1 ml of blocking buffer prior to staining. Blocking buffer consisted of a base buffer of PBS with 0.3% BSA and 50 µg/ml gentamicin (PBS/BSA) to which 0.01% NaN<sub>3</sub> (Sigma Chemical Corp., St. Louis MO) and 5% mouse serum (Gemini BioProducts, Woodland, California, USA) were added. The polyclonal antibodies and monoclonal antibodies (mAbs)

conjugated with FITC, PE, and PC5 used to characterize the cell surface and intracytoplasmic markers on the fetal liver cells are listed in Table 1.

Cells were separated into aliquots of  $5 \times 10^5$  cells/well in 96 well V-bottom plates (Costar, Cambridge, Mass, USA). Cell surface staining was done with PE-conjugated and PC5-conjugated mAbs for 30 minutes. Cells were then washed with PBS/BSA at  $130 \times g$  for 2.5 minutes. Cells then underwent intracytoplasmic staining by fixing in 1% paraformaldehyde in PBS overnight at 4°C. Triton X-100 (Sigma, St. Louis, USA) was then added to final concentration of 0.1% and the cells were allowed to stand for 40 minutes at 4°C. They were then washed with PBS, and once again with PBS with 0.1% triton and 1% BSA. Intracytoplasmic staining was then performed with FITC-conjugated antibodies; mouse IgG<sub>2a</sub>, anti CK7/8, or anti- $\alpha$ -smooth muscle actin (SMA); in PBS with 0.1% triton and 1% BSA for 30 minutes at room temperature. Cells were washed twice in PBS with 0.1% triton and 1% BSA and then suspended in PBS with 0.1% paraformaldehyde for flow cytometric analysis. A two-step technique was used when staining cells with anti-desmin and mouse IgG<sub>2b</sub>. Cells were initially labeled for intracytoplasmic desmin with unconjugated anti-desmin for 30 minutes and then washed twice, were after they were stained with PE-conjugated goat anti-mouse antibody. Cells were then washed twice and analyzed by flow cytometry using either a single laser FACScan or dual laser FACSCalibur flow cytometer (BD Biosciences).

#### *Cell culture.*

CD34<sup>+</sup>CD38<sup>-</sup> cells and CD34<sup>+</sup>NGFR<sup>+</sup>CD45<sup>-</sup> cells were cultured in HepatoSTIM (BD Biosciences, San Jose, California, USA) or Dulbecco's modified Eagle's medium (DMEM) (Mediatech, Herndon, Virginia, USA) with 5% fetal bovine serum (FBS). Cells were cultured in standard tissue-culture treated culture wells (BD Biosciences) as well as on Biocoat Cellware



(Becton Dickinson, Bedford, Mass, USA) coated with Matrigel or fibronectin. Growth factors used during culture included combinations of 10 ng/ml hepatocyte growth factor (HGF, Peprotech Inc., Rocky Hill, NJ, USA), 10 ng/ml basic fibroblast growth factor (bFGF, Peprotech Inc.), 10 ng/ml acidic fibroblast growth factor (aFGF, Peprotech Inc.), 20 ng/ml epidermal growth factor (EGF, R&D Systems, Minneapolis, MN, USA), 20 ng/ml vascular endothelial growth factor (VEGF, R&D systems), 10 ng/ml oncostatin M (OSM, Peprotech Inc.), 20 ng/ml granulocyte-macrophage colony-stimulating factor (GM-CSF, R&D Systems) and 50 ng/ml kit ligand (KL, R&D Systems).

## Results

### *Detection of CD34<sup>+</sup>CK7/8<sup>+</sup> cells in the fetal liver.*

Ten fetal livers were analyzed for the presence of CD34<sup>+</sup>CK7/8<sup>+</sup> cells, i.e. candidate common stem cells for the hepatic and hematopoietic lineages. CD34<sup>+</sup>CK7/8<sup>+</sup> cells were present at a frequencies ranging from 3.4 to 8.5%. These cells had a high forward and side light-scatter profile (Fig. 1, R2), indicating a relatively larger size and cytoplasmic complexity than most hematopoietic cells found in the fetal liver. An adjacent population of CD34<sup>-</sup>CK7/8<sup>+</sup> cells was also observed (Fig. 1, R1), which had a similar light scatter profile as the CD34<sup>+</sup>CK7/8<sup>+</sup> cells. Two distinct populations of CD34<sup>+</sup> cells were observed, those staining for CK7/8 (Fig. 1, R2), and those not staining for CK7/8 (Fig. 1, R3). The latter population represents hematopoietic progenitors and stem cells previously described, which are characterized by a low side-light scatter and low to moderate forward-light scatter (18, 20, 21). These findings indicate that CD34<sup>+</sup>CK7/8<sup>+</sup> cells are a distinct population of cells from any hematopoietic precursors previously characterized.

### *Analysis of CK7/8 expression on hematopoietic stem cells and progenitors in the fetal liver.*

Over  $1.5 \times 10^6$  fetal liver cells, with a low side-light scatter and low to moderate forward-light scatter, were analyzed to determine whether CK7/8 is expressed by hematopoietic precursors. Negligible CK7/8 expression was detected among CD34<sup>+</sup>CD38<sup>-</sup> stem cells (Fig. 2, R3) and CD34<sup>+</sup>CD38<sup>+</sup> committed hematopoietic-progenitors (Fig. 2, R2). CD34<sup>low</sup>CD38<sup>+</sup> cells had a small population of CK7/8<sup>+</sup> cells. Similar phenotypic findings were observed in all of our experiments.

*Culture of hematopoietic stem cells in the presence of hepatopoietins.*

To test if CD34<sup>+</sup>CD38<sup>-</sup> stem cells can generate hepatocytes, these cells were grown under various culture conditions in the presence of combinations of the growth factors HGF, EGF, VEGF, aFGF, bFGF, OSM, GM-CSF and KL. These growth factors were chosen because they are known to support the growth and/or differentiation of hepatocytes and or hematopoietic stem cells (22-26). CD34<sup>+</sup>CD38<sup>-</sup> cells demonstrated little growth over 3 weeks, unless GM-CSF and/or KL were added to the cultures. GM-CSF and KL are known to support hematopoiesis and in the presence of these cytokines large numbers of non-adherent cells were generated. In some cultures, a few adherent cells were observed that morphologically resembled hepatocytes (Fig. 3A and B), but the possibility that these cells could be hematopoietic in origin could not be excluded. Analysis of which cytokine was responsible for the presence of the adherent cells indicated that VEGF supported the generation of the adherent cell population. Large numbers of adherent cells were generated in cultures containing VEGF+KL (Fig. 3B) and VEGF+GM-CSF+KL. The adherent cells expressed CD45, CD14 and HLA-DR, indicating that they were hematopoietic cells rather than hepatocytes (data not shown). Therefore, our findings indicate that CD34<sup>+</sup>CD38<sup>-</sup> cells fail to generate cells other than hematopoietic cells under any of the culture conditions that we have tested.

*CD34<sup>+</sup>CK7/8<sup>+</sup> cells express markers associated with stellate cells.*

The CD34<sup>+</sup>CK7/8<sup>+</sup> cells with a high forward and light scatter profile were further characterized using a panel of cell surface and intracytoplasmic markers (Table 1). They stained positive for CD13, CD59, desmin, and NGFR. The rest of the phenotypic panel was mostly negative, including the hematopoietic and hepatic stem-cell markers CD90 and CD117 (Table 1). Representative results of the phenotypic analysis of CD34<sup>+</sup>CK7/8<sup>+</sup> cells are shown in Fig. 4.

Low expression of the hematopoietic progenitor marker, CD38, was observed and very low expression of the common leukocyte antigen, CD45, was seen. In addition, lymphocyte markers (CD1a, CD2, CD3, CD4 and CD40), monocyte/granulocyte markers (CD11b, CD11c, CD14, and CD33), and dendritic cell markers (CD80 and CD83) were not expressed by CD34<sup>+</sup>CK7/8<sup>+</sup> cells. These findings indicate that CD34<sup>+</sup>CK7/8<sup>+</sup> cells are not hematopoietic cells.

CD34<sup>+</sup>CK7/8<sup>+</sup> cells did express CD13, CD59, and NGFR (Table 1 and Fig. 4). NGFR, within the liver, has been found on stellate cells (27, 28), which prompted us to further investigate desmin expression, an intracytoplasmic protein found in hepatic stellate cells (29). CD34<sup>+</sup>CK7/8<sup>+</sup> cells did express desmin (Table 1). Examination of CD34<sup>+</sup> cells with (SMA) mAb, another stellate cell marker (30), revealed a similar pattern of expression as for CK7/8<sup>+</sup> (Table 2). Furthermore, CD34<sup>+</sup>NGFR<sup>+</sup>CD45<sup>-</sup> cells were isolated and cultured in DMEM with 5% FBS and were found to adhere to fibronectin coated plates. No growth was observed over a 3 week period, but cells with a stellate appearance were observed as shown in Fig. 3C and 3D.

## Discussion

CD34<sup>+</sup>CK7/8<sup>+</sup> cells have been hypothesized to represent a population of common stem cells for hepatic and hematopoietic tissues in the human fetal liver (6). Our aim was to better define these cells based on physical characteristics and cell surface/intracytoplasmic protein expression. Our findings indicate that these cells are not progenitor cells of either the hepatic or hematopoietic systems, but rather they are hepatic stellate cells.

CD34<sup>+</sup>CK7/8<sup>+</sup> cells are conspicuously different from hematopoietic stem and progenitor cells being larger in size and having a more complex cytoplasm. Moreover, staining for the common leukocyte antigen, CD45, as well as a panel of hematopoietic cell markers was consistently negative, a strong indication that these cells are not of hematopoietic origin. Although CD34 is a well characterized marker of hematopoietic precursors, some non-hematopoietic cells also express this marker (17, 18, 31, 32). Consistent with our findings, CK7/8 has previously not been found in hematopoietic cells (9). Cytokeratins 7 and 8 are intermediate filament proteins that interact with keratin 19 and 18, respectively. CK 8/18 can be found in hepatocytes biliary cells, stellate cells and adult hepatic progenitor cells. CK 7/19 are located on biliary and adult hepatic progenitor cells. The possibility that CD34<sup>+</sup>CK7/8<sup>+</sup> cells represent a type of hepatocyte progenitor was not indicated by our analysis. CD34<sup>+</sup>CK7/8<sup>+</sup> cells lacked CD90 (Thy-1) and CD117 (c-kit) expression, which differentiates these cells from previously described hepatic oval cells (4, 7), as well as hematopoietic stem cells (18, 20). Lemmer et al. observed low CD117 expression on some CD34<sup>+</sup> fetal liver cells but CK7/8 expression wasn't examined in conjunction with these markers (6). It is likely the CD117<sup>+</sup>CD34<sup>+</sup> cells observed by these authors were hematopoietic precursors (18, 20). We did observe a low number of CK7/8<sup>+</sup> cells among CD34<sup>+</sup> cells with the light-scatter profiles of

hematopoietic precursors, which may have been due to non-specific binding of the CAM5.2 mAb. Furthermore, cultures of hematopoietic precursors failed to provide any conclusive evidence that these cells could form hepatocytes.

Markers observed on or within the CD34<sup>+</sup>CK7/8<sup>+</sup> cells included CD13, CD59, NGFR, desmin and SMA, which taken together strongly suggests that these cells are stellate cells. CD34<sup>+</sup>CK7/8<sup>+</sup> cells expressed high levels of CD13, aminopeptidase N, which is a membrane bound metalloprotease found on endothelial cells (33) and hematopoietic cells (34, 35). Since a small percentage of CD34<sup>+</sup>CK7/8<sup>+</sup> cells expressed the endothelial cell marker CD31, the possibility that some endothelial cells are among the CD34<sup>+</sup>CK7/8<sup>+</sup> population exists. However, most CD34<sup>+</sup>CK7/8<sup>+</sup> cells are likely to be stellate cells, although we are not aware of any reports regarding the expression of CD13 on stellate cells. CD59, protectin, is a glycoprotein that inhibits cell lysis by the membrane attack complex of the complement system. It is widely distributed throughout the human body (36) and in the fetal liver expression of CD59 begins at 6 weeks gestation (37). CD59 is distributed among both endothelial and hematopoietic cells but not on hepatocytes (38). CD34<sup>+</sup>CK7/8<sup>+</sup> cells expressed desmin, which is widely expressed on non-hematopoietic cells in the liver during development. Only once the liver is fully mature, however, is desmin localized primarily in stellate cells (29). Expression of desmin on CD34<sup>+</sup>CK7/8<sup>+</sup> cells indicated that these cells were either ductal plate or stellate cells (29). Hepatic stellate cells have been shown to be an source of several neurotrophins and express neurotrophin receptors (27, 28, 39). Although hepatocytes may express low levels of NGFR (40), no other liver cell has been reported to have high expression. The function of NGFR on stellate cells is unknown. Expression of NGFR on stellate cells is hypothesized to support tissue growth, differentiation and/or migration of these cells (27). Further evidence that the CD34<sup>+</sup>CK7/8<sup>+</sup> cells

are stellate cells was the expression of intracytoplasmic SMA by CD34<sup>+</sup> cells with the same phenotypic characteristics as CD34<sup>+</sup>CK7/8<sup>+</sup> cells. SMA expression in conjunction with desmin expression is characteristic of stellate cells within the adult liver (41). We isolated CD34<sup>+</sup>NGFR<sup>+</sup>CD45<sup>-</sup> cells, which did not appear to proliferate within the growth medium over a 3-week period although they did adhere to the fibronectin coated wells. The morphology of these cells was consistent with that of stellate cells.

Despite evidence that hematopoietic stem cells can contribute to the development of hepatocytes in experimental and transplant settings, a natural link between the hematopoietic and hepatic systems during human development has not been demonstrated. Although our findings do not support CD34<sup>+</sup>CK7/8<sup>+</sup> cells being common hepatic/hematopoietic stem cells, further study of the fetal liver may yet uncover common stem cells. Failure of CD34<sup>+</sup>CD38<sup>-</sup> hematopoietic stem cells to form hepatocytes in culture may simply result from our lack of understanding as to the requirements of hepatopoiesis and should not be viewed as conclusive evidence that these cells cannot form hepatocytes. The developmental relationships of stellate cells, hematopoietic, hepatic and endothelial cell lineages warrants further study. In this regard it is worth mention that a few CK7/8<sup>+</sup> cells were observed among low side light-scatter, CD34<sup>low</sup> cells and that the CD34<sup>+</sup>CK7/8<sup>+</sup> population appeared to arise from a CD34<sup>-</sup>CK7/8<sup>+</sup> population of cells with low to moderate side light scatter properties. These observations suggest a pathway of stellate cell development that has precursors with similar phenotypic properties as hematopoietic precursors.

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**Table 1. Expression of cell-surface and intracytoplasmic markers by CD34<sup>+</sup>CK7/8<sup>+</sup> cells.**

Specificity	Clone	Source <sup>1</sup>	Cell Type <sup>2</sup>	% Expression <sup>3</sup>	Specificity	Clone	Source <sup>1</sup>	Cell Type <sup>2</sup>	% Expression <sup>3</sup>
CD1a	BL6	BC	DCs	0 %	CD45	2D1	BD	Hematopoietic cells	11 %
CD2	T11-RD1	BC	T cells, NK cells	3.4 %	CD45RO	VCHL1	BC	T cells, Monocytes, HSCs	1.7 %
CD3	SK7	BD	T cells	6.5 %	CD45RA	L48	BD	T cells, B cells, Monocytes, HPCs	3.2 %
CD4	Leu-3a	BD	T cells, Myelocytes	5.7 %	CD56	C5.9	Exalpa	NK cells, T cells	0.6 %
CD7	CD7-6B7	Caltag	T cells, NK cells, HPCs	0.9 %	CD59	MEM43	Caltag	Most cell types	94 %
CD8	SK1	BD	T cells, NK cells	0 %	CD64	32.2	Caltag	Monocytes	7.9 %
CD10	W8E7	BD	B cells	4.4 %	CD71	YDJ1.2.2	BC	HPCs	4.0 %
CD11a	MEM-25	Caltag	Leukocytes	0.8 %	CD80	MAB104	BC	B cells, T cells	0.5 %
CD11b	CR3	Caltag	Myelocytes, NK cells	2.2 %	CD83	HB15a	BC	B cells, T cells, DCs	2.1 %
CD11c	5-HCL-3	BD	Myelocytes	0.3 %	CD86	HA5.2B7	BC	Monocytes, B cells, DCs	2.9 %
CD13	L138	BD	Myelocytes, HPCs, HSCs, ECs	98.4 %	CD90	5E10	BD	HSCs, Mesenchymal cells, ECs	6.7 %
CD14	V1MC6	Caltag	Monocytes	7.5 %	CD94	HP-3B1	BC	T cells, NK cells	3.2 %
CD16	3G8	Caltag	NK cells, Myelocytes	0 %	CD95	DX2	BD	Leukocytes, HPCs, HSCs, Hepatocytes	3.8 %
CD18	MEM-48	Caltag	Leukocytes	0.5 %	CD116	SC06	BC	Myelocytes, HPCs, HSCs	0.9 %
CD19	SJ2SC1	BD	B cells	1.2 %	CD117	104D2	BD	HPCs, HSCs	6.8 %
CD20	HI47	Caltag	B cells	0 %	CD122	p75	BD	T cells, NK cells	3.2 %
CD25	CD25-3G10	Caltag	Lymphocytes	3.6 %	CD123	9F5	BD	Myelocytes, HPCs, HSCs	0 %
CD31	1F11	BC	Leukocytes, HPCs, HSCs, ECs	13.1 %	CD124	S4-56C9	BC	T cells, B cells, HPCs	5.2 %
CD33	P67.7	BD	Myelocytes, HPCs, HSCs	2.9 %	CD126	M91	BC	B cells, HPCs	3.7 %
CD38	HB7	BD	Leukocytes, HPCs	7.9 %	CD127	R34.34	BC	T cells, Monocytes, HPCs	2.1 %
CD40	Mab98	BC	B cells, Monocytes, DCs, EpiCs	3.6 %	CD135	SF1.340	BC	HPCs, HSCs	8.3 %
CD42	MB45	Caltag	Megakaryocytes	0 %	Anti-NGFR	C40-1457	BD	Stellate Cells	84%
CD44	MEM85	Caltag	Hematopoietic cells, Mesenchymal cells, ECs	7.2 %	Anti-Desmin	RD301	BD	Stellate Cells	98.2 %

<sup>1</sup>Company Abbreviations: Caltag Laboratory (Burlingame, CA, USA), Beckman-Coulter (BC) (Marseille, France), BD Biosciences (BD) (San Jose, Ca, USA), Exalpha (Boston, Massachusetts, USA), R&D System (Minneapolis, Minn.,USA), and Sigma Chemical Corporation (St. Louis, Missouri, USA)

<sup>2</sup>The likely cell types in the fetal liver to express each of the studied markers are indicated. Abbreviations: dendritic cells (DCs), hematopoietic progenitor cells (HPCs), hematopoietic stem cells (HSCs), endothelial cells (ECs), basal epithelial cells (EpiCs)

<sup>3</sup>Results are reported as the mean of 2 to 5 experiments performed on separate tissues.



**Table 2. Expression of cell-surface and intracytoplasmic markers by CD34<sup>+</sup>SMA<sup>+</sup> cells.**

<b>Specificity</b>	<b>Clone</b>	<b>% Expression<sup>1</sup></b>	<b>Specificity</b>	<b>Clone</b>	<b>% Expression<sup>1</sup></b>
CD13	L138	98.1 %	CD59	MEM43	96.2 %
CD33	P67.7	4.7 %	CD90	5E10	4.2 %
CD38	HB7	12.9 %	CD117	104D2	2.1 %
CD40	Mab98	1.4 %	Anti-NGFR	C40-1457	92.3 %
CD45	2D1	7.7 %	Anti-Desmin	RD301	94.1 %

<sup>1</sup>Results are reported as the mean of 2 experiments performed on separate tissues.

## Figure Legends

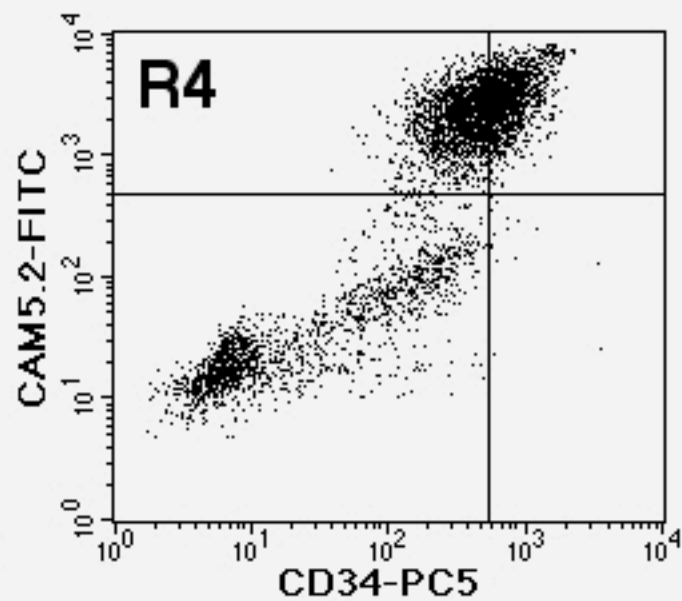
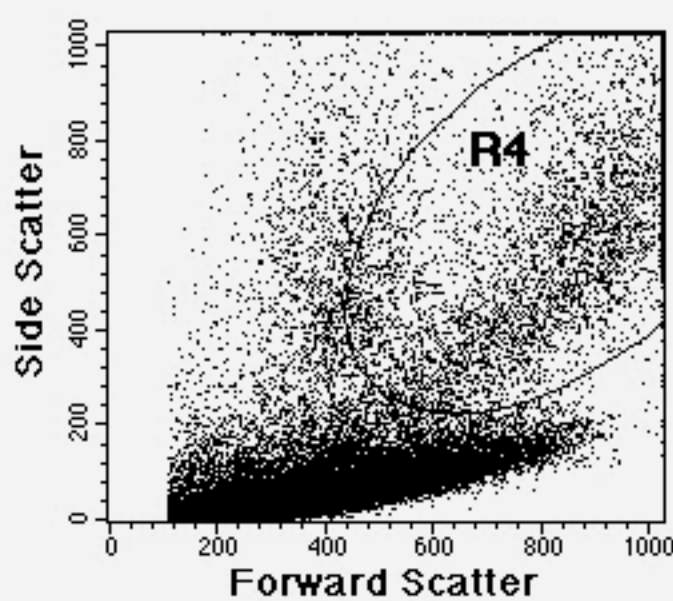
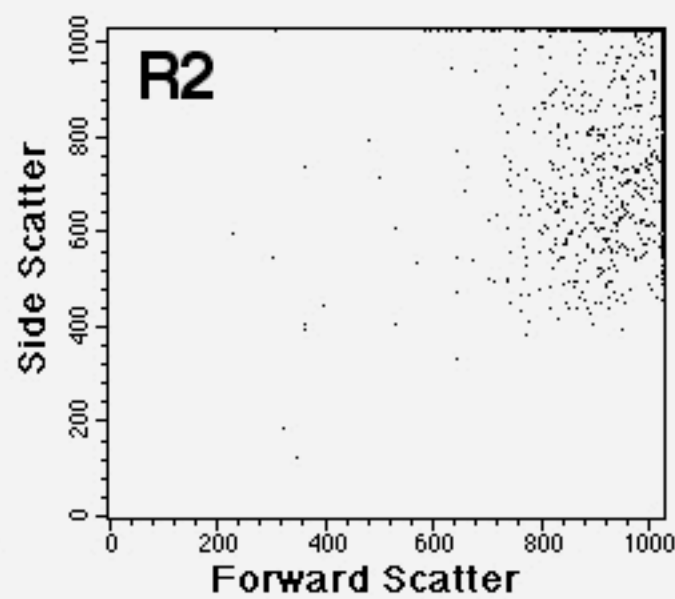
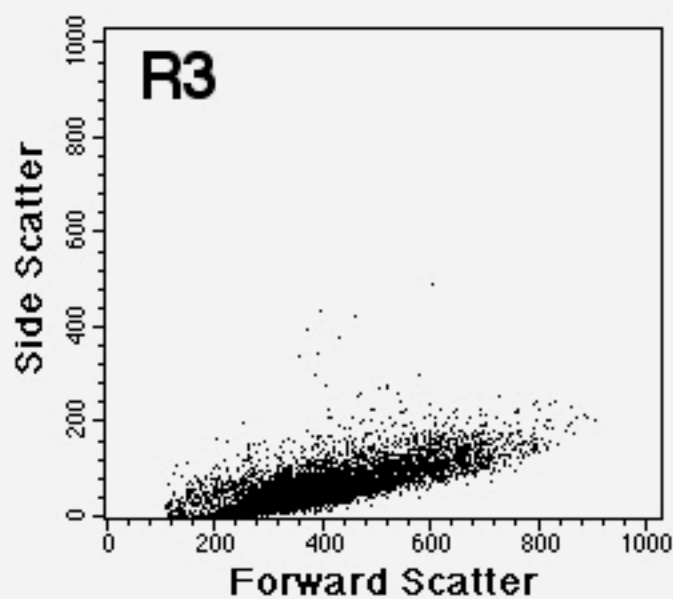
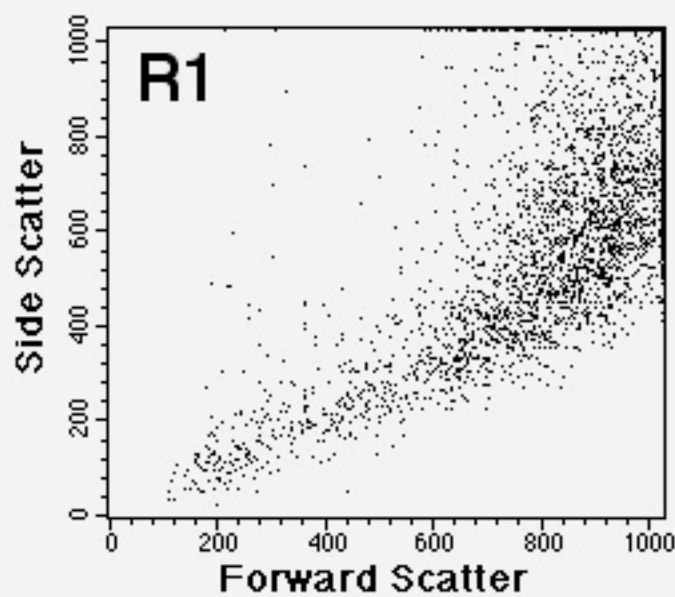
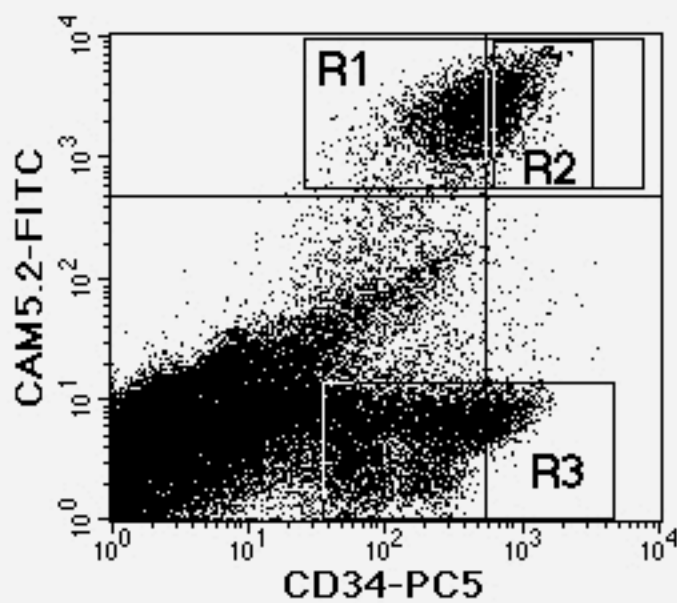
Figure 1. Expression of CD34 and CK7/8 by distinct subpopulations of cells in the human fetal liver. Morphologic comparison of 3 subpopulations of cells which represent the proposed hematopoietic/hepatic stem cells, CD34<sup>-</sup>CAM5.2<sup>+</sup> (R1) and CD34<sup>+</sup>CAM5.2<sup>+</sup> (R2), and the hematopoietic stem/progenitor cells CD34<sup>+</sup>CAM5.2<sup>-</sup> cells (R3). In comparison to the lower forward and side scatter in the known hematopoietic stem cell region (R3), CD34<sup>-/+</sup>CAM5.2<sup>+</sup> (R1 & R2) have a higher side and forward scatter indicating a larger more cytoplasmically complex cell. These cells also have a much higher level of background autofluorescence compared to cells found in R3 (data not shown). This is evident using a region (R4) to select high forward and side scatter events, which enriches for CAM5.2<sup>+</sup> cells. Quadrant markers were drawn based on a controls and gating on R4. Intracytoplasmic staining of CK7/8 was done with mAb CAM5.2.

Figure 2. CK7/8 expression by hematopoietic precursors. Hematopoietic progenitors and stem cells within the human fetal liver were defined by their low side- and forward-light scatter (oval region) and by expression of CD34 (rectangular regions). Hematopoietic precursors were subdivided into 3 progressively more immature subpopulations: CD34<sup>low</sup>CD38<sup>+</sup> mature progenitors (R1), CD34<sup>+</sup>CD38<sup>+</sup> early progenitors (R2) and CD34<sup>+</sup>CD38<sup>-</sup> stem cells (R3). Intracytoplasmic staining of CK7/8 and by control antibody is shown for each subpopulation. Over  $1.5 \times 10^6$  CD235a<sup>-</sup> cells were analyzed.

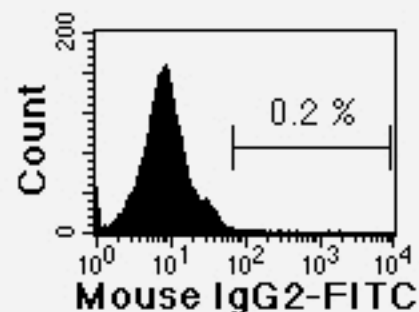
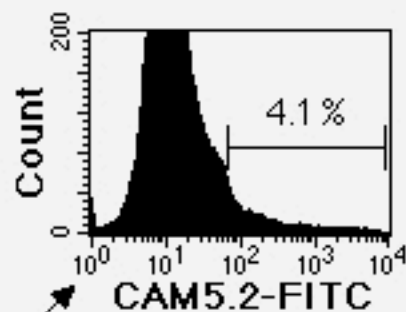
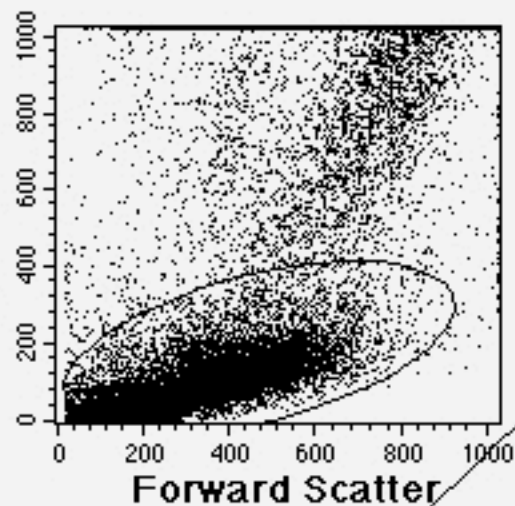
Figure 3. Morphology of cultured CD34<sup>+</sup>CD38<sup>-</sup> and CD34<sup>+</sup>NGFR<sup>+</sup>CD45<sup>-</sup> cells. CD34<sup>+</sup>CD38<sup>-</sup> hematopoietic stem cells were grown in HGF+VEGF+OSM (A), which resulted in little growth over a 3 week period. Some large adherent cells were observed (arrows), but were too few to ascertain if they were hepatocytes. Similarly, adherent cells were observed when

CD34<sup>+</sup>CD38<sup>-</sup> cells were cultured in VEGF+KL (B). Large adherent cells were generated (arrows), but were identified as myeloid cells (data not shown). Isolated CD34<sup>+</sup>NGFR<sup>+</sup>CD45<sup>-</sup> cells cultured in DMEM with 5% FBS had a stellate morphology (arrows, C and D).

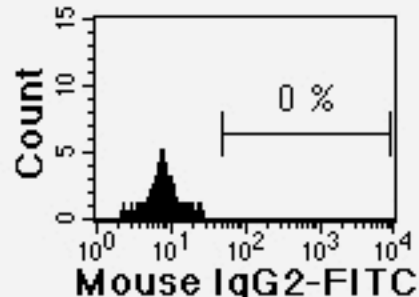
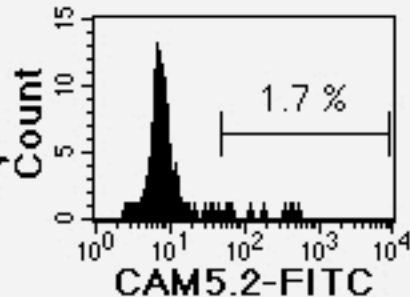
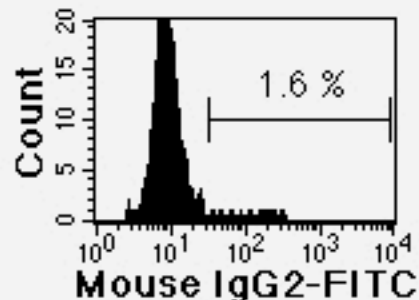
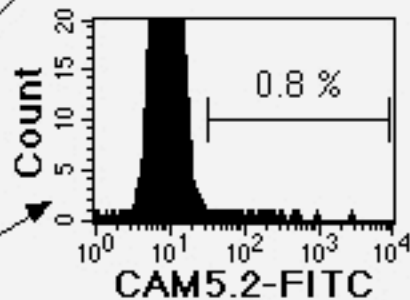
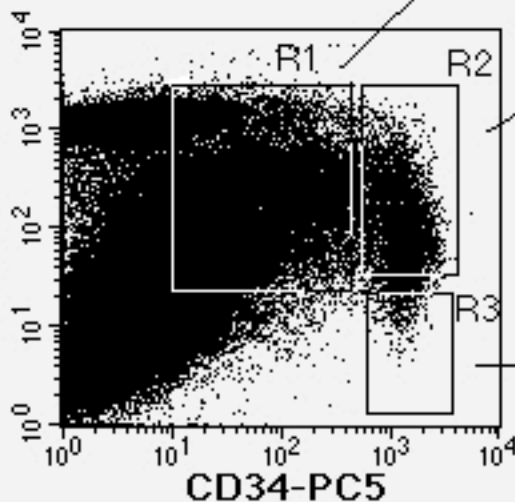
Figure 4. Phenotypic analysis of CD34<sup>+</sup>CK7/8<sup>+</sup> cells. CD34<sup>+</sup>CK7/8<sup>+</sup> cells were gated using the two regions shown and analyzed for the expression of various cell surface markers. Background staining on CD34<sup>+</sup>CK7/8<sup>+</sup> cells is shown in the top right histogram plot.

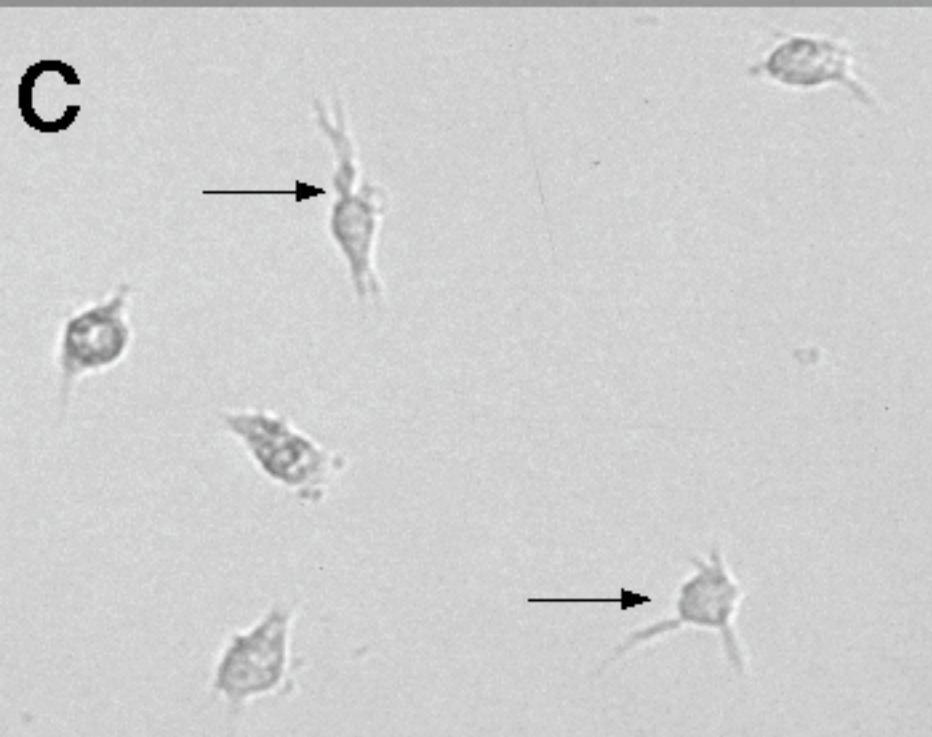
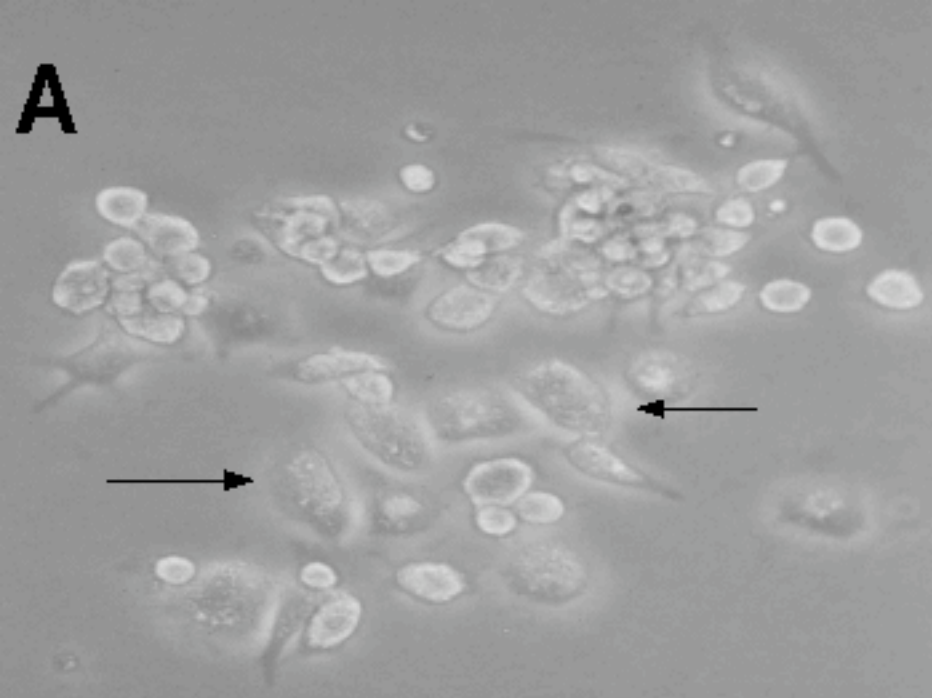


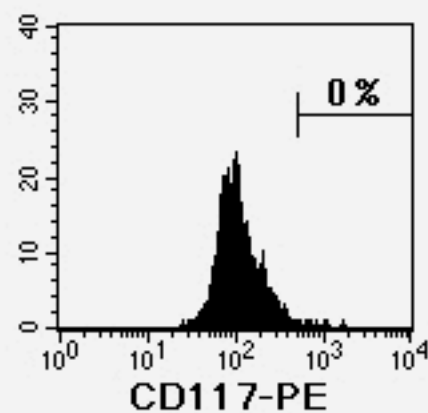
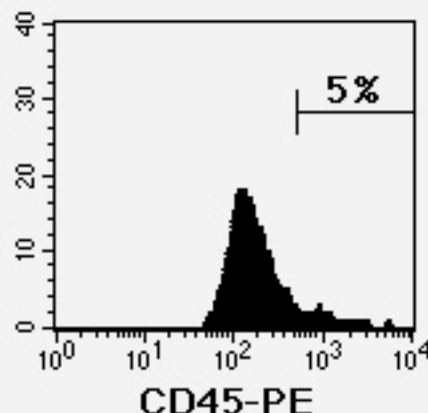
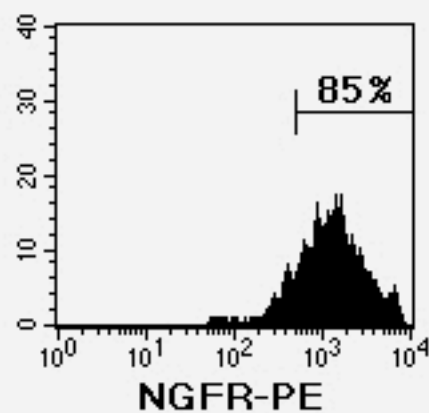
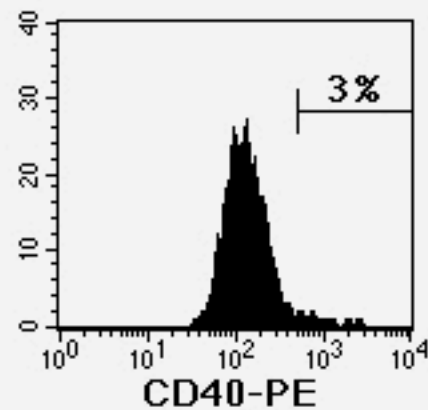
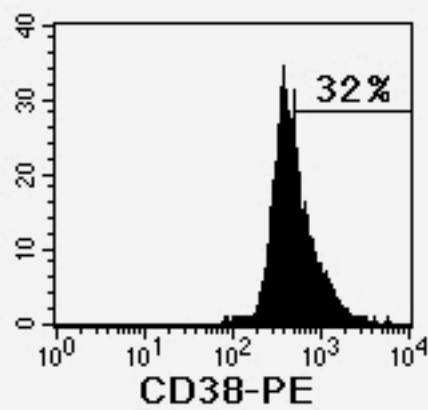
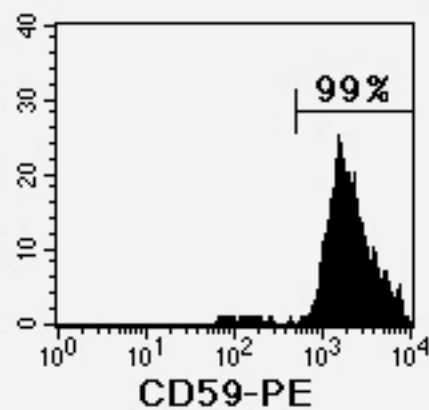
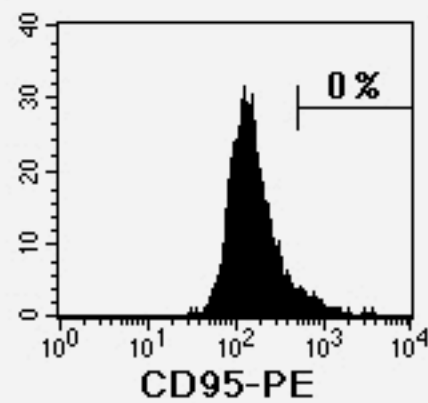
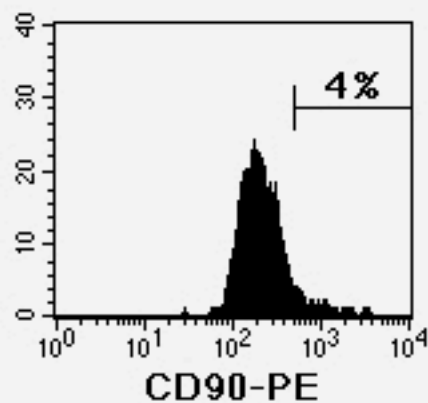
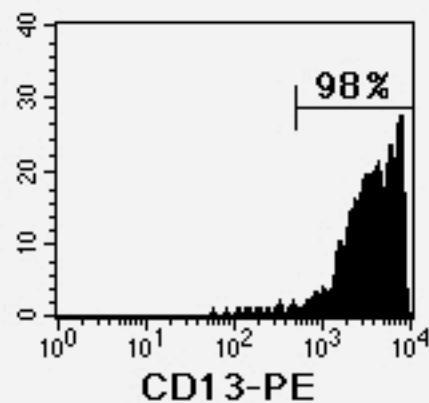
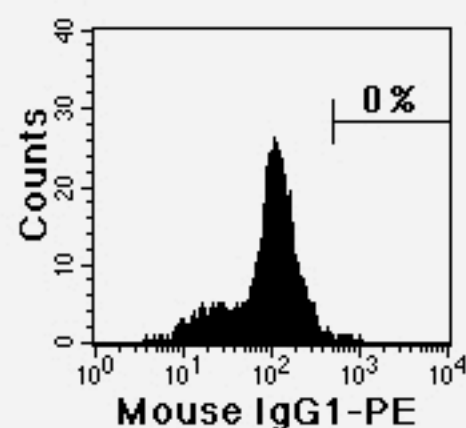
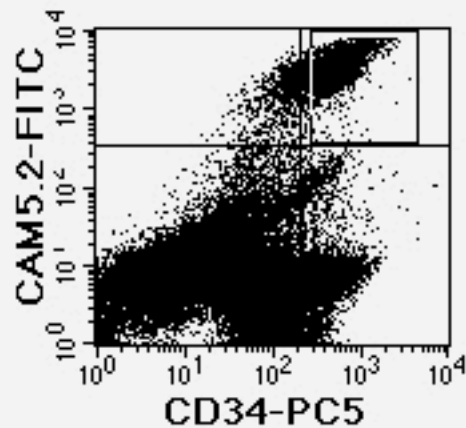
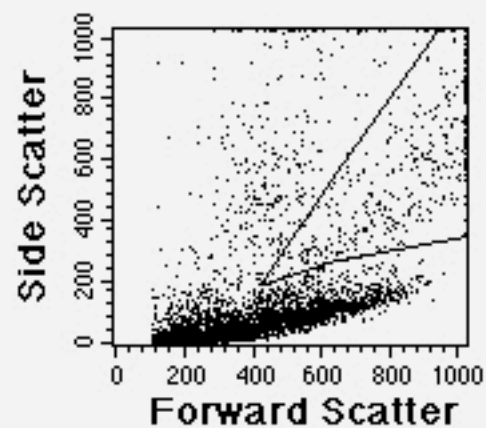
Side Scatter



CD38-FITC







Counts

