Human CD34$^+$ cells differentiate into microglia and express recombinant therapeutic protein

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In rodents, bone marrow-derived cells enter the brain during adult life. Allogeneic bone marrow transplantation is used to treat genetic CNS diseases, but the fate of human bone marrow and CD34$^+$ cells within the brain remains to be elucidated. The present study demonstrates that cells derived from human CD34$^+$ cells, isolated from either cord blood or peripheral blood, migrate into the brain after infusion into nonobese diabetic/severe combined immunodeficient mice. Both types of CD34$^+$-derived cells differentiate into perivascular and ramified microglia. The lentiviral transfer of genes into CD34$^+$ cells before infusion does not modify the differentiation of human CD34$^+$ cells into microglia, allowing new transgenic proteins to be expressed in these cells. The transplantation of CD34$^+$ cells could thus be used for the treatment of CNS diseases.

Up to 20% of the total nonneuronal cell population is made of microglia (1). Microglia are ubiquitously distributed in the CNS and play a major role in the response to infectious, traumatic, inflammatory, and ischemic processes, as well as in degenerative CNS diseases, such as Alzheimer's disease, multiple sclerosis, or Parkinson's disease. The adult brain contains two subsets of microglia: the resting microglia, which ramify throughout the brain parenchyma, and the perivascular microglia, which resemble peripheral macrophages (1, 2).

Following a long debate about their origin, microglia are now believed to be derived from bone marrow as liver, spleen, or lung macrophages (2). Murine bone marrow-derived cells enter the CNS and differentiate into microglia (3–5). In mice, the turnover of perivascular microglia reaches 30% 1 year after engraftment, whereas the turnover of ramified microglia is much slower (6, 7). However, the subset of bone marrow-derived cells that are the progenitors of microglia has not been characterized. In mice, transplantation of transduced bone marrow cells allows the expression of glucocerebrosidase or GFP in microglia (8, 9). In humans, there are very little data documenting the fate of bone marrow-derived cells in the CNS after bone marrow transplantation (BMT) (10, 11). However, that BMT is used to treat genetic CNS diseases like Hurler disease or X-linked adrenoleukodystrophy (ALD) (12, 13) suggests that bone marrow cells may serve as vehicles for the delivery of genes into the human CNS.

Transplantation of CD34$^+$ hematopoietic cells is replacing that of whole bone marrow cells for many applications, including autotransplantation in cancer, non-HLA genoidentical BMT, and gene therapy (14–16). Human CD34$^+$ cells can be easily collected from cord blood at birth or from peripheral blood after cytokine mobilization. We studied the migration, differentiation, and distribution of human CD34$^+$ cells purified either from umbilical cord blood (UCB) or from mobilized peripheral blood (MPB) in the brain of nonobese diabetic/severe combined immunodeficient (NOD/SCID) mouse. Our results demonstrate that a fraction of these cells, when infused into NOD/SCID mice, differentiate into both perivascular and parenchymal microglia and allow the expression of new transgenic proteins throughout the brain.

Materials and Methods

Isolation of Human CD34$^+$ Cells. CD34$^+$ cells were isolated from UCB of normal individuals or from granulocyte colony-stimulating factor MPB from ALD patients after obtaining their informed consent and in accordance with approved institutional guidelines. CD34$^+$ cells were purified by immunomagnetic selection (17). Fluorescence-activated cell sorting (FACS) analysis performed on a FACStar (Becton Dickinson) showed that the CD34$^+$ population had a purity of over 90%. The CD34$^+$ cells were then stored in liquid nitrogen until used.

Transduction of UCB or MPB CD34$^+$ Cells. Human UCB and MPB CD34$^+$ cells were transduced according to previously described procedures (17, 18) with a self-inactivating lentiviral/HIV vector (TRIPΔU3) carrying expression cassettes for the human ALD protein or the enhanced GFP (EGFP) under the control of the elongation factor 1α promoter. Briefly, CD34$^+$ cells were incubated with lentiviral vector in the presence of four recombinant human cytokines (stem cell factor, Flt3-ligand, IL-3, pegylated-megakaryocyte growth and differentiation factor; UCB = 100/10/100/60 ng/ml, MPB = all 10 ng/ml). Lentiviral vector was added twice and conducted for a period of 36 h (MPB) or 72 h (UCB). Production and infectious titration of ALD and EGFP lentiviral vectors were done as described (19). Virus titers were 10^9 units/ml. Monocytes/macrophages were identified by using monoclonal anti-CD68 KP1 (DAKO) (17). Transduction efficiency in CD45$^+$ or CD68$^+$ cells was tested by determining the percentage of EGFP- and ALD-positive cells by FACS or immunocytochemistry (17).

Transplantation into NOD/SCID Mice. UCB (10^5) or 1.5×10^6 native or transduced MPB CD34$^+$ cells were intravenously injected into 92 sublethally irradiated NOD-LtSz-*scid*/*scid* (NOD/SCID) mice [3 Gy, at 0.43 Gy/min; in a Philips (Eindhoven, The Netherlands) x-ray RT250 irradiator].

Monitoring of Human Engraftment. Bone marrow cells were harvested from recipient mice, and the presence of human cells was checked in individual mice by FACS by using mouse anti-human CD45-FITC monoclonal antibody (Immunotech, Villepinte-Roissy CDG, France) (17, 18).

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Abbreviations: NOD/SCID, nonobese diabetic/severe combined immunodeficient; BMT, bone marrow transplantation; UCB, umbilical cord blood; MPB, mobilized peripheral blood; EGFP, enhanced GFP; IHC, immunohistochemistry; ALD, adrenoleukodystrophy; VLCFA, very long-chain fatty acid; FACS, fluorescence-activated cell sorting; ANA, antinuclear antibody; RCA-1, ricinus communis agglutinin-1.

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Analysis of Human Cell Engraftment in the CNS. Animals were killed 18 weeks after transplantation. Deeply anesthetized NOD/SCID mice were perfused with PBS, followed by 4% paraformaldehyde for the mice transplanted with EGFP-transduced UCB cells. Brains were removed, frozen in isopentane, and stored at −80°C until analysis. Serial sections (10 μm thick) were cut at –17°C by using a cryostat, fixed in 4% formaldehyde for 10 min, and permeabilized in 0.1% PBS-Triton X-100 and every 22nd section was collected for analysis. In preliminary experiments, the number of donor cells was determined by *in situ* hybridization (ISH) detecting human Alu sequences and fluorescent immunohistochemistry (IHC) visualizing human-specific nucleus marker [antinuclear antibody (ANA)] on six adjacent identical brain sections of six NOD/SCID mice. Then, the number of donor cells was analyzed by IHC alone. ISH was performed by using a human-specific Alu oligodeoxynucleotide probe labeled in 5′ with digoxigenin (20). After being denaturated at 75°C for 20 min, brain slides were prehybridized in wet steam-room chambers at 45°C for 90 min. Slides were then placed overnight at 45°C in the hybridization solution containing the Alu probe (0.02 pmol/μl). After five washes, an antibody against digoxigenin conjugated to alkaline phosphatase (dilution 1:2,000, Roche Diagnostics) was added, and digoxigenin was revealed by using nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP) (Promega). The IHC identification of human cells was performed by using a primary mouse anti-human nuclei monoclonal antibody (ANA) (dilution 1:10, Chemicon, Temecula, CA), specific for all human cell types.

To identify microglial, neuronal, and astrocytic differentiation of human CD34$^+$-derived cells, brain sections were double-labeled with ANA visualized with FITC or Cy3-conjugated anti-mouse IgG (H+L) secondary antibody (dilution 1:200, Jackson ImmunoResearch) and (*i*) biotinylated ricinus communis agglutinin-1 (RCA-1) (dilution 1:400, Vector Laboratories) followed by direct labeling with FITC or Cy3-conjugated streptavidin (Jackson ImmunoResearch). RCA-1 lectin binds to β-D-galactose residues and labels microglial cells in adult human brain (21); or rabbit anti-ionized calcium binding adapter molecule 1 (Iba1) antibody (dilution 1:400, Imai, Japan (22). Iba1 is specifically expressed in cells of the monocyte/macrophage lineage, including microglia. (*ii*) mouse anti-NeuN antibody (dilution 1:500, Chemicon); or mouse anti-βIII-tubulin (dilution 1:600, Covance, Princeton, NJ); or rabbit antineurofilament 68-kD antibody (dilution 1:100, Chemicon). (*iii*) rabbit antiglial fibrillary acidic protein (GFAP) antibody (dilution 1:200, DAKO). The expression of EGFP and human ALD protein was visualized by using a rabbit anti-EGFP antibody [dilution 1:1,000, Abcam, UK) and a rabbit anti-ALD protein antibody (dilution 1:4,000 (23)]. The expression of mouse ALD protein was studied by using a specific rabbit anti-ALD protein antibody [dilution of 1:1,000, (24)].

Brain sections were incubated overnight with primary antibodies at 4°C. All primary antibodies were diluted in PBS-Triton X-100, 0.1% with 500 μg/ml goat IgG, and all subsequent incubations or washes were done in 0.1% PBS–Triton X-100. Rabbit primary antibodies were revealed with a Cy3 or FITC-conjugated anti-rabbit IgG (H+L) antibody (dilution 1:100, Chemicon).

Double labeling using anti-NeuN or -βIII-tubulin and ANA monoclonal antibodies was performed by using the TSA Plus Fluorescence systems (DuPont NEN, Boston). ANA was used at a dilution of 1:300 to be undetectable after incubation with Cy3-conjugated anti-mouse IgG(H+L) antibody (Jackson ImmunoResearch), used to reveal NeuN or βIII-tubulin. ANA was then visualized after incubation with FITC-conjugated anti-mouse antibody and then with an anti-FITC-horseradish peroxisidase conjugate to amplify the signal via FITC-tyramide.

Cell nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI). Appropriate filters for each or combined fluorochrome were used on a light microscope equipped for fluorescence (Nikon E800, Eclipse). The colocalization of markers was confirmed on selected slides by z-stacking analysis of images collected with a laser scanning confocal microscope (Bio-Rad MRC 1024).

Analysis of Very Long-Chain Fatty Acid (VLCFA) Concentrations in Hematopoietic Cells. CD34$^+$ cells were seeded in methylcellulose and macrophage colonies (colony-forming units–macrophages) were assessed 15 days after for VLCFA analysis. VLCFA were extracted and measured by gas/liquid chromatography/mass spectrometry (23). Results are expressed as C26:0/C22:0 ratios.

Results

Human UCB or MBP CD34$^+$-Derived Cells Migrate into the Brain of NOD/SCID Mice After Peripheral Infusion. We injected 10^5 human UCB and 1.5×10^6 MPB CD34$^+$ cells, respectively into 35 and

Table 1. Engraftment and microglial differentiation of human CD34$^+$-derived cells in the bone marrow and brain of NOD/SCID mice

CD34$^+$ cell origin	Percent of human cells (CD45$^+$) in bone marrow	Number of human cells (ANA$^+$)/section ± SD*	Number of human microglia (ANA$^+$/RCA$^+$)/section ± SD*	Percent of microglia in all human cells†	Percent of human microglia in total microglia‡
UCB	5	16 ± 2	15.7 ± 2.1	98.1	0.54
	50	60 ± 24	59.8 ± 21	99.1	2.06
	62	58 ± 3	57.3 ± 0.6	98.7	1.97
	71	118 ± 16	113.3 ± 16	96	3.90
	75	100 ± 19	98.5 ± 19.5	98.2	3.39
	80	137 ± 31	136.3 ± 27	98.7	4.70
	81	94 ± 26	92 ± 27	98.2	3.17
	83	137 ± 19	136.8 ± 18.3	99.6	4.71
	90	106 ± 14	101 ± 10	95.2	3.48
	90	114 ± 2	113 ± 2.4	99.1	3.89
MPB	7	16 ± 6.8	15.7 ± 6.5	96.3	0.54
	10	12 ± 4.6	11.5 ± 4.3	96.6	0.39
	25	14 ± 6.3	14.2 ± 6.3	100	0.49
	64	25 ± 6.9	25 ± 6.9	100	0.86
	75	86 ± 9.5	84.7 ± 9.7	98.8	2.92

*Values are the mean ± SD of counts performed on 25 sections.
†Percentage of (ANA$^+$/RCA$^+$)/ANA$^+$ cells.
‡Percentage of (ANA$^+$/RCA$^+$)/number of total microglial cells per section.

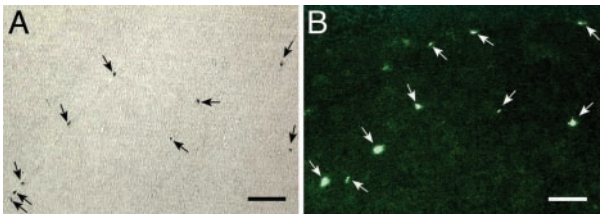

Fig. 1. Engraftment of human CD34$^+$ cells in the brain of NOD/SCID mice. The human origin of the engrafted cells was established by using *in situ* hybridization with an Alu probe (*A*) or by immunohistochemistry by using the ANA marker (*B*). (Bars = 100 μm.)

29 sublethally irradiated NOD/SCID mice. Eighteen weeks after the transplantation, human hematopoietic engraftment was analyzed by FACS of bone marrow cells with anti-human CD45 antibody. The level of engraftment remained inferior to 5% in 49 mice, which were excluded from further study. Fifteen mice with engraftment ranging from 5% to 90% were studied for the presence of human cells in the brain (listed in Table 1), by *in situ* hybridization detecting human Alu sequences, and fluorescent IHC visualizing human-specific nucleus marker (ANA).

In six NOD/SCID mice (see *Materials and Methods*), we found an average of 89 (SD $\pm$ 20) Alu- and 96 (SD $\pm$ 14) ANA-positive cells per brain section ($r = 0.96$) (Fig. 1). ANA- or Alu-positive cells were detected after PBS perfusion, indicating that bone marrow-derived cells were an integral part of the brain parenchyma. 4′,6-Diamidino-2-phenylindole (DAPI) counterstaining to highlight the nucleus confirmed the exclusive nuclear localization of the ANA marker. IHC with ANA was then used to study brain engraftment in the remaining ($n = 9$) transplanted mice. Sixteen to 137 ANA-positive cells were detected per brain section in 10 mice transplanted with UCB CD34$^+$ cells and 12–86 in five mice transplanted with MPB CD34$^+$ cells (Table 1). The number of ANA-positive cells per brain ranged from 13,200 to 150,700 and correlated very closely ($r = 0.87$) with the percentage of systemic bone marrow engraftment (Fig. 2). The number of human cells detected in the brain of transplanted mice after UCB or MPB CD34$^+$ cells transplantation was thus comparable.

Human UCB or CD34$^+$-Derived Cells Differentiate into Microglia. ***Phenotype of engrafted cells.*** IHC revealed that 95–100% of ANA-positive cells in mouse brain expressed the RCA-1 lectin (Fig. 3 *A* and *B* and Table 1). This lectin is expressed markedly in perivascular microglia and weakly in parenchymal microglia. Fifty percent of ANA-positive cells also expressed the Iba1 antigen (Fig. 3 *C* and *D*). ANA- and Iba1-positive cells had an

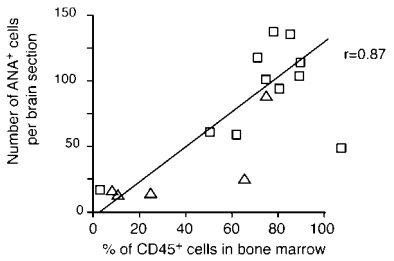

Fig. 2. Cerebral homing of human CD34$^+$-derived cells is correlated to bone marrow engraftment of UCB (□) or MPB (△) CD34$^+$ cells. Human cell engraftment was assessed by scoring the number of human CD45$^+$ cells in the bone marrow of NOD/SCID mice, 18 weeks after transplantation. Brain engraftment was assessed by scoring the number of ANA-positive cells in the brain of recipient mice at the same time. In two animals, the brain engraftment was limited despite high levels of bone marrow engraftment.

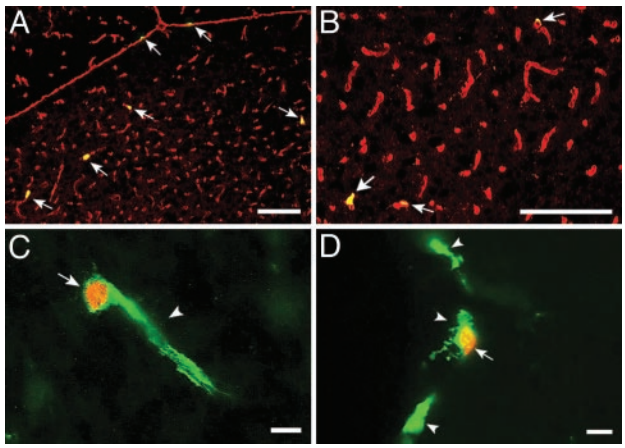

Fig. 3. Differentiation of human CD34$^+$-derived cells in the brain of NOD/SCID mice. (*A* and *B*) Double-labeling of brain sections identifies cells coexpressing the microglial marker RCA-1 (in red, Cy3) and the human ANA marker (in green, FITC). Yellow staining (arrows) indicates colocalization of both markers in the same cell. (*C* and *D*) Double-labeling of brain sections identifies cells coexpressing the microglial marker Iba1 (in green, FITC, arrowheads) and the ANA marker (in red, Cy3, arrows) in their nuclei.

elongated shape (Fig. 3*C*) or displayed the ramified morphology characterizing resting or nonactivated microglia (2) (Fig. 3*D*). This demonstrates that a fraction of human CD34$^+$-derived cells differentiate in cells harboring markers of the two main subsets of microglia. None of the ANA-positive cells were labeled with a specific antibody that recognizes the mouse ALD protein, normally expressed in mouse microglia (data not shown). It is therefore unlikely that CD34$^+$-derived cells acquired the phenotype of microglia as a result of spontaneous cell fusion with mouse microglia (25, 26).

No ANA-positive cells were found to express glial fibrillary acidic protein (GFAP). Only 0.03% ANA-positive cells (3 of 10,000 cells counted in the brain of four NOD/SCID mice transplanted with UCB CD34$^+$ cells) were stained with anti-NeuN, anti-βIII-tubulin, and antineurofilament 68-kDa antibodies (data not shown). This indicates that differentiation of human CD34$^+$ cells in neurons was a rare event.

Number and brain distribution of microglia derived from human CD34$^+$ cells. The total number of RCA-1-positive cells of human origin per brain section was 2,900 $\pm$ 300, $\approx$5% of mice brain microglia (Table 1). Engrafted cells were found in all of the brain but predominantly in the cerebral cortex, cerebellum, colliculus, and olfactory bulbs (Fig. 4*A*). ANA/RCA-1 or ANA/Iba1 cells were also detected in the hippocampus, hypothalamus, striatum, brainstem, and white matter (corpus callosum, fimbria, and cerebellum). No difference was observed between ANA/RCA-1-positive cells deriving from human UCB or MPB CD34$^+$ cells. The same percentage of ANA-positive cells was found in the brain parenchyma and the perivascular sites, whatever the origin of CD34$^+$ cells (Fig. 4 *B–D*). Less than 1% of ANA-positive cells were present in the choroid plexus of the lateral ventricles.

Microglia Derived from Genetically Modified Human CD34$^+$ Cells Express Transgenic Proteins. Ten NOD/SCID mice were infused with UCB CD34$^+$ cells transduced *ex vivo* with a lentiviral vector driving the expression of the EGFP cDNA under the control of the elongation factor 1α promoter. Eight of 10 transplanted mice were engrafted in proportions ranging from 30% to 70%. Eighteen weeks after transplantation, EGFP was expressed in 90–98% of human bone marrow (CD45$^+$) cells (Fig. 5*A*), indicating efficient and stable gene transfer and expression, as already shown with this lentiviral vector (18). We analyzed the

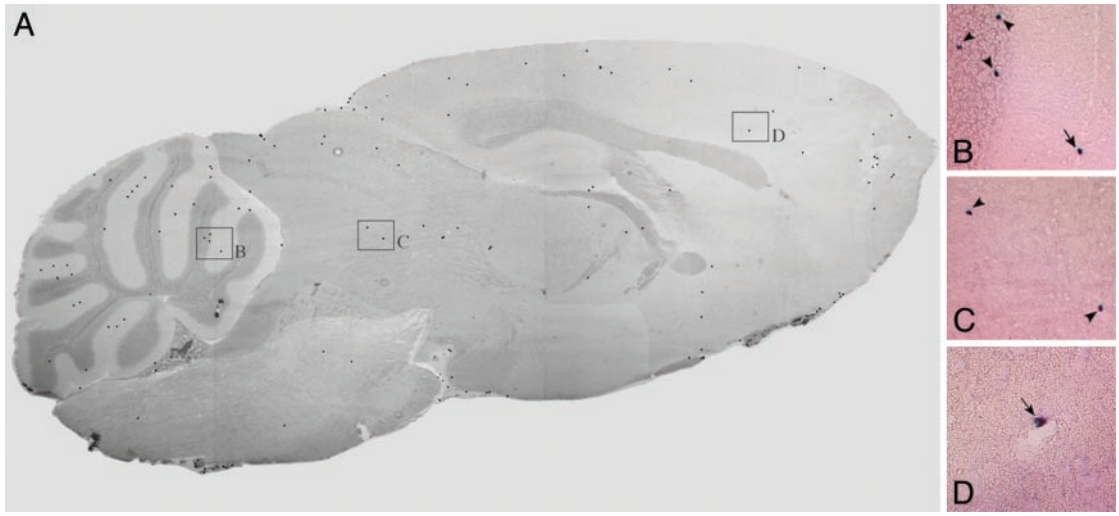

Fig. 4. Distribution of human CD34$^+$-derived cells in the brain of NOD/SCID mice. (*A*) Human-engrafted cells, detected by *in situ* hybridization with Alu probe, were widely distributed throughout the brain. One hundred forty-one human cells are detected on this brain section. This section is representative of the brain of mice transplanted with human UCB CD34$^+$ cells (≥80% of engraftment) (see Table 1). (*B–D*) Large magnification. Engrafted cells were located at perivascular sites as well as in the brain parenchyma (*B*, cerebellum; *C*, colliculus; *D*, cortex). Perivascular (*B* and *D*, arrows) and intraparenchymatous (*B* and *C*, arrowheads) engrafted cells were identified on the basis of morphology and proximity to or absence of nearby blood vessels.

brain of four mice with 50–68% engraftment. Eighty-three percent to 96% of ANA-positive cells identified in the brain of these four mice expressed recombinant EGFP, suggesting no significant gene silencing in the CNS. Nonramified and ramified EGFP-expressing cells were abundant in the cerebral cortex, cerebellum, and colliculus (Fig. 5 *B* and *C*). EGFP-positive cells expressed the monocyte/macrophage-specific antigen Iba1 (Fig. 5*D*). ANA-positive cells and Iba1-positive cells expressing EGFP were detected in perivascular sites and parenchyma with number and distribution comparable to that of untransduced UCB CD34+ cells, indicating that the *ex vivo* transduction process did not alter the ability of these cells to enter the brain and differentiate into microglia.

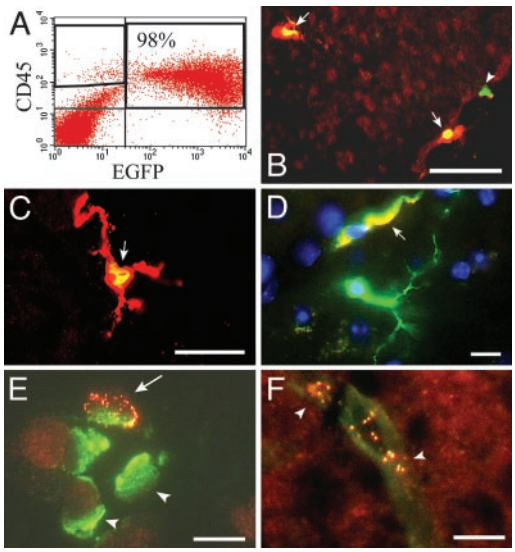

Fig. 5. Genetically modified human CD34$^+$-derived microglial cells express marker or therapeutic genes in the brains of NOD/SCID mice. (*A*) Dot-plot analysis showing the percentage of human CD45$^+$ cells expressing EGFP in mouse bone marrow. (*B* and *C*) EGFP-transduced UCB CD34$^+$-derived cells in mouse brain. Nonramified (*B*) and ramified (*C*) cells express EGFP (in red, Cy3) and the human ANA marker (green, FITC) yielding a yellow staining in their nuclei (arrows). One ANA-positive cell (*B*, arrowhead) does not express EGFP. (*D*) Human UCB CD34$^+$-derived cells differentiate into microglia expressing EGFP. Colocalization of EGFP (in red, Cy3) and Iba1 (in green, FITC) results in yellow staining (arrow). (*E*) CD68$^+$ macrophages (in green, FITC, arrowheads) expressing recombinant ALD protein (punctate staining in red, Cy3, arrow) are present in the bone marrow of transplanted mice. (*F*) MPB CD34$^+$ cells from ALD patient engrafted in mouse brain express recombinant ALD protein (punctate staining in red, Cy3, arrows) and the microglial RCA-1 lectin (in green, FITC). [Bars = 100 μm (*B*), 25 μm (*C*), and 10 μm (*D–F*).]

We also studied the migration of genetically modified human MPB CD34$^+$ cells in the X-linked ALD model (X-ALD). X-ALD is a lethal demyelinating genetic disorder that can be corrected by allogenic BMT (13, 27). VLCFA accumulate in cells and tissues from ALD patients. The ALD protein, an ATP-binding cassette transporter located in peroxisomal membrane, plays an unknown role in the metabolism of VLCFA. MPB CD34$^+$ cells from two ALD patients were transduced with a lentiviral vector driving the expression of human ALD cDNA under the control of the elongation factor 1α promoter. Cells were infused into 18 NOD/SCID mice. Eighteen weeks after transplantation, human ALD protein was expressed in 30–85% of human bone marrow cells, including macrophages (Fig. 5*E*) from six engrafted (3–75%) mice. Cells expressing recombinant ALD protein and the RCA-1-lectin were detected in the brain of all engrafted mice, and detailed analysis was performed in mice showing the highest percentage of bone marrow engraftment ($n = 4$). As observed after transplantation of unmodified UCB or MPB CD34$^+$ cells, cells expressing the recombinant ALD protein and the RCA-1 lectin were detected in perivascular and parenchymal sites (Fig. 5*F*). The cerebral distribution of ALD protein-positive cells was comparable to that of ANA/RCA-1-positive cells. Ten percent of ANA/RCA-1-positive cells derived from transduced MPB ALD CD34$^+$ cells expressed recombinant human ALD protein. Because NOD/SCID mice do not accumulate VLCFA, it was not possible to demonstrate the functionality of the ALD protein *in vivo*. However, before the infusion of transduced MPB ALD CD34$^+$ cells, the functionality of the ALD protein was demonstrated in CD34$^+$cells and in derived macrophages (colony-forming units–macrophages) by measuring the correction of VLCFA accumulation (Table 2). These data indicate that genetically modified human CD34$^+$

Table 2. Correction of VLCFA metabolism in ALD CD34$^+$ cells and in derived macrophages (colony-forming units–macrophages) after transduction

Type of cell	Observed C26:0/C22:0 ratio			Percent of transduced MPB ALD cells expressing ALD protein*	Percent of biochemically corrected cells†
	Control cells (UCB CD34$^+$ cells)	MPB ALD CD34$^+$ cells			
		Nontransduced	Transduced		
CD34$^+$ cells	0.041 ± 0.018 (n = 4)	0.182 (n = 1)	0.127 (n = 1)	40	39
Macrophages (CFU-M)	0.042 ± 0.016 (n = 2)	0.121 (n = 1)	0.072 (n = 1)	45	63

n, Number of analyzed samples; CFU-M, colony-forming units–macrophages.
*Percentage of ALD-positive cells/1,000 counted cells.
†The percentage of biochemical correction is calculated by linear regression from C26:0/C22:0 values observed in nontransduced and transduced cells.

cells can differentiate into microglia and express a protein of therapeutic interest in the brain after peripheral infusion.

Discussion

Our data demonstrate that a fraction of human CD34$^+$ cells differentiate to form a substantial number of ramified microglial cells. Unlike perivascular microglia, thought to be periodically replaced during adult life by cells derived from bone marrow, as are peripheral tissue macrophages, resting microglia, which are ramified, were considered to be a permanent population. Our results were observed in the absence of any cerebral lesion, but we cannot exclude that standard whole-body irradiation could facilitate the migration, engraftment, and differentiation of a subset of CD34$^+$-derived cells in ramified microglia. We do not know whether these human CD34$^+$ progenitor cells penetrate directly in the brain after infusion, or whether they need to home first in the bone marrow. Due to experimental limitations, it is impossible to demonstrate that the cells derived from human CD34$^+$ cells, which are found in the brain of transplanted NOD/SCID mice, have acquired true microglial functions. However, these cells expressed the Iba1 marker specific for ramified microglia.

The number of human CD34$^+$-derived microglial cells found in mice brain correlated with the percentage of CD34$^+$ cell engraftment in the bone marrow of transplanted animals. Engraftment of MPB CD34$^+$ cells was less efficient than that of UCB CD34$^+$ cells in the NOD/SCID model (28, 29), because 15 times more MPB cells were needed to achieve similar engraftment in bone marrow than UCB cells. However, MPB and UCB human CD34$^+$ cells exhibited identical capacities as progenitors of perivascular and ramified microglial cells. This has important implications for therapeutic use. If autotransplantation is to be considered, UCB CD34$^+$ cells must be stored at birth, whereas MPB can be collected lifelong.

A few studies have suggested that mouse bone marrow cells could follow differentiation into neurons and glia (3–5). Other reports raised doubts about the developmental plasticity of adult hematopoietic stem cells (30, 31). Our data indicate that, in the NOD/SCID model, neuronal differentiation of human CD34$^+$ cells is certainly a rare event during the first 18 weeks after transplantation. In normal mice, neuronal differentiation of murine bone marrow cells was reported to be a late event observed only 6 months after transplantation (32, 33). The limited life span of NOD/SCID mice did not allow us to address this issue.

The lineage of human CD34$^+$ cells into microglia should *a priori* allow the delivery of therapeutic genes within the human CNS, as shown with the ALD gene. The lentiviral transfer of ALD and EGFP genes into human CD34$^+$ cells did not modify the capacity of derived cells to cross the blood–brain barrier, engraft in the CNS, and differentiate into perivascular or ramified microglia. The expression of ALD or GFPs in mice brain was maintained during several weeks.

Autotransplantation of genetically modified human CD34$^+$ cells provides an approach to the treatment of genetic CNS disorders. Functions not normally performed by microglia could be generated. In addition, human CD34$^+$-derived microglia would likely be attracted to sites of neuronal damage (9).

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