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Mobilized Human CD34+ Hematopoietic Stem Cells Enhance Tumor Growth in a Nonobese Diabetic/Severe Combined Immunodeficient Mouse Model of Human Non-Hodgkin's Lymphoma[1]

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ABSTRACT

Autologous peripheral blood stem cell mobilization is increasingly applied in the treatment of hematological malignancies. Despite the frequent clinical use in a setting of residual disease, it is not known whether mobilization of hematopoietic stem cells might facilitate tumor outgrowth *in vivo*. In the bone marrow, a bipotential precursor for hematopoietic and endothelial cells called hemangioblast exists. This hemangioblast, characterized by the expression of CD34 and vascular endothelial growth factor receptor (VEGFR)-2, is released from the bone marrow by mobilization and might be able to result in not only the generation of peripheral blood cells but vasculogenesis due to differentiation of the hemangioblast along the endothelial lineage [in addition to VEGFR-2 expression, angiopoietin-2 (ANG-2) expression can also be found in this stage]. New vessel formation in the tumor is critical for tumor growth. A xenotransplant model was established with 10×10^6 Daudi cells (non-Hodgkin's lymphoma) s.c. injected in the neck region of nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice, who were sublethally irradiated with 2 Gy. At day 10 after tumor inoculation, half of the mice were given 0.5×10^6 human CD34+ cells i.v., whereas the other half were given PBS i.v. The human CD34+ cells were obtained from leukapheresis samples of myeloma patients undergoing autologous peripheral blood stem cell mobilization. We compared tumor growth and human-specific VEGFR-2 and ANG-2 expression in the two groups. Tumor growth is enhanced 2-fold when mobilized hematopoietic human CD34+ cells are given compared with PBS controls ($P = 0.004$). In addition, the human-specific VEGFR-2 and ANG-2 reverse transcription-PCR was only positive in the tumors of mice i.v. injected with human CD34+ cells. This indicates that the injected human CD34+ cells home to the tumors and differentiate along the endothelial lineage. In the present study, we demonstrate that mobilized human CD34+ hematopoietic cells injected i.v. might facilitate the outgrowth of tumors in the setting of minimal residual disease. Malignant tumors are capable of incorporating human CD34+ hematopoietic cells. This study questions the safety of leukapheresis in patients with (residual) tumor and has important implications for further development of intensive chemotherapy protocols with autologous stem cell rescue.

INTRODUCTION

Autologous transplantation with mobilized hematopoietic progenitor cells is increasingly applied in the treatment of malignant diseases, such as lymphoma, leukemia, and neuroblastoma (1–5). The use of peripheral blood mobilized stem cells is often preferred above bone marrow due to a more rapid hematological recovery after transplantation (6, 7). Bone marrow MNCs[3] including CD34+ cells can be mobilized to the peripheral blood by pretreatment with hematopoietic growth factors including G-CSF, granulocyte macrophage colony-stimulating factor, or IL-8 (4, 8, 9). CD34+ cells comprise approximately 1–4% of the bone marrow MNCs and <0.05% of peripheral blood MNCs. However, treatment with growth factors, such as G-CSF, can increase the percentage of CD34+ cells in the peripheral blood up to 5% (4, 8, 9). Despite the frequent clinical use of mobilization in a setting of (minimal) tumor, it is not known whether mobilization influences tumor growth.

Asahara *et al.* (10) and Takahashi *et al.* (11) reported that CD34+ endothelial progenitors have the potential to differentiate *in vitro* to endothelial cells and can be incorporated *in vivo* into sites of neoangiogenesis. Even stronger evidence was provided by Shi *et al.* (12), who demonstrated that bone marrow-derived CD34+ endothelial progenitors had the capacity to line an implanted vascular prosthesis. More recently, emerging data show that a subset of human CD34+ cells coexpressing VEGFR-2 and AC133 have phenotypic features of endothelial cell progenitors playing a role in postnatal angiogenesis (13, 14).

The process of new vessel formation plays a critical role in solid tumor outgrowth, invasion, and metastasis (15, 16). Recently, in hematological malignancies, neovascularization has also been observed, especially in lymphoma, multiple myeloma, acute lymphoblastic leukemia and acute myeloid leukemia (17–22).

The process of neovascularization has long been considered as angiogenesis (vessel sprouting from preexisting vessels; Ref. 16). However, more recent studies have indicated that postnatal vasculogenesis implying *in situ* vessel formation from CD34+ hematopoietic stem cells might occur due to the expansion and differentiation of a specific subset of hematopoietic VEGFR-2-expressing stem cells called hemangioblasts to (precursor) endothelial cells that are also able to express ANG-2 (10, 23). Evidence indicates that ANG-2 plays a critical role in vessel outgrowth, remodeling, and maturation (24, 25).

Based on these results, we hypothesized that the infused mobilized CD34+ cells might facilitate the outgrowth of tumor or residual tumor. We demonstrated that (*a*) i.v. infused human CD34+ mobilized cells enhance tumor growth in a xenotransplantation mouse NOD/SCID model of human non-Hodgkin's lymphoma and (*b*) mobilized human CD34+ cells are incorporated in the tumor and differentiate along the endothelial lineage.

MATERIALS AND METHODS

Cell Line/Cells. The Daudi cell line is a well-characterized B lymphoblast cell line (phenotype CD3−, CD14−, CD10+, CD19+, CD20+) derived from

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[3] The abbreviations used are: NON/SCID, nonobese diabetic/severe combined immunodeficient; MNC, mononuclear cell; VEGFR, vascular endothelial growth factor receptor; VEGF, vascular endothelial growth factor; ANG-2, angiopoietin-2; RT-PCR, reverse transcription-PCR; G-CSF, granulocyte colony-stimulating factor; IL, interleukin; bFGF, basic fibroblast growth factor; MMP, matrix metalloproteinase.

a male with Burkitt's lymphoma (kindly provided by Dr. C. Melief, Leiden University Medical Center, Leiden, the Netherlands; Ref. 26). The cells were cultured in Iscove's media supplemented with 2 mM L-glutamine, 10% FCS, 100 units/ml penicillin, 100 μg/ml streptomycin, and 50 μM β-mercaptoethanol.

After obtaining informed consent, peripheral blood MNCs were obtained from leukapheresis samples of multiple myeloma patients ($n = 3$) undergoing autologous peripheral blood stem cell mobilization. Cells were collected by apheresis during the regeneration phase after high-dose cyclophosphamide (6 g/m^2) in the presence of G-CSF (5 μg/kg). CD34+ cells were isolated by positive selection using Isolex-300 (Baxter, Deerfield, IL) according to the manufacturer's instructions. At the end of the procedure, CD34+ cell purity was reanalyzed by flow cytometry using anti-HPCA-2 and was >90%. No CD138+ (BB4) cells were detected in stem cell collections.

Animal Study. NOD/SCID mice (kindly provided by Dr. L. D. Shultz, The Jackson Laboratory, Bar Harbor, ME) were bred and maintained at the Central Animal Facility, University of Groningen. Animals were kept under laminar flow conditions during the experiment. Our model was generated by s.c. injecting 10×10^6 Daudi cells into the neck region of sublethally irradiated (2 Gy) 6–8-week-old NOD/SCID mice. The mice were evaluated for tumor growth. At day 11 after tumor inoculation, human CD34+ cells were injected in the tail vein, and control mice received PBS. To control for outgrowth of myeloma, mice were injected with mobilized human CD34+ cells (3×10^6 cells) after 3 Gy of irradiation. Tumor-bearing mice were killed by carbon dioxide asphyxiation, and tumor engraftment was confirmed by histological and immunohistochemical studies. All procedures involving animals were done in accordance with local ethical animal laws and policies.

Phenotypic Analysis. Antihuman CD34, CD38, CD10, CD33, CD56, CD7, and anti-GPA phycoerythrin-1- or FITC-conjugated antibodies were purchased from Becton Dickinson (Mountain View, CA), and antihuman CD138 was obtained from Immunoquality Products (Groningen, the Netherlands). Biotinylated rat antimouse CD18 (PharMingen, San Diego, CA) developed with streptavidin-allophycocyanin was used to differentiate between mouse and human cells. Cells (10^5) were labeled with FITC- and/or phycoerythrin-1-conjugated antibodies or isomatched controls for 30 min on ice. Subsequently, the cells were washed in PBS containing 1% BSA and 0.1% NaN_3. Cells were analyzed on a FACScan flow cytometer using CellQuest software from Becton Dickinson.

Immunohistochemical Stainings. Tumor tissue and other used tissues from mice were collected and snap-frozen until analysis. Tumor specimens were cut in 4-μm sections and fixed in acetone. Slides were treated with different antibodies diluted in PBS/1% BSA. An appropriate peroxidase-labeled second antibody was used, followed by the addition of substrate for peroxidase. The following antibodies (obtained from PharMingen) were used: (*a*) rabbit antihuman FVIII (dilution, 1:3200; DAKO AS, Glostrup, Denmark); (*b*) rabbit antihuman IgM (dilution, 1:4000); (*c*) mouse antihuman CD20 and CD22 (dilution, 1:1000); and (*d*) rat antimouse CD31 (0.5 mg/ml). As secondary antibodies, we used peroxidase-labeled swine antirat IgG (dilution, 1:40) and biotin-labeled swine antirabbit $(Fab')_2$ (dilution, 1:300; both from Southern Biotechnology Associates, Birmingham, AL). Streptavidin peroxidase-labeled second step reagent (1:300) was obtained from DAKO.

RT-PCR Analysis for Several Angiogenic Factors. Total RNA from tumor tissues was extracted by the Trizol method, following the manufacturer's instructions (Life Technologies, Inc., Grand Island, NY). cDNAs were prepared by reverse transcription at 37°C for at least 1 h in a 20-μl reaction mixture containing 2 μg of total RNA, random hexamers (Pharmacia), 5× first strand buffer, RNasin, and 1 μl of reverse transcriptase (Life Technologies, Inc.). cDNA was amplified in the presence of primers, 10× buffer, 1.5 mM $MgCl_2$, deoxynucleotide triphosphates, and Taq (Life Technologies, Inc.). The mixture was amplified in a Perkin-Elmer apparatus with PCR cycle conditions specific for the PCRs tested. PCR product was analyzed by electrophoresis in a 1.5% agarose gel. Gels were stained with ethidium bromide and photographed. Specific primers for β_2-microglobulin were CCA-GCA-GAG-AAT-GGA-AAG-TC (sense) and GAT-GCT-GCT-TAC-ATG-TCT-CG [antisense; PCR product, 268 bp; 22 cycles; T_{ann} (annealing temperature) 55°C]. Specific primers for IL-8 were TTG-GCA-GCC-TTC-CTG-ATT (sense) and AAC-TTC-TCC-ACA-ACC-CTC-TG (antisense; PCR product, 247 bp; 28 cycles; T_{ann} 55°C; 1.25 mM $MgCl_2$ and 5% DMSO). Specific primers for VEGF were GAG-TGT-GTG-CCC-ACT-GAG-GAG-TCC-AAC (sense) and CTC-CTG-CCC-GGC-TCA-CCG-CCT-CGG-CTT (antisense; PCR products, 177, 312, and 384 bp; 30 cycles; T_{ann} 60°C). The primers for VEGF span the splice junctions, allowing the amplified product of each splice variant to be separated electrophorically. Specific primers for bFGF were [GCC-TTC-CCG-CCC-GGC-CAC-TTC-AAG-G (sense) and GCA-CAC-ACT-CCT-TTG-ATA-GAC-ACA-A (antisense; PCR product, 180 bp; 34 cycles; T_{ann} 56°C], and for restriction analysis of the PCR product, *Afl*III (mouse specific; 60 and 115 bp) and *Alu*I (human specific; 54 and 124 bp) were used. For ANG-2 [PCR product, 234 bp; 34 cycles; T_{ann} 55°C (R&D Systems)], *Sca*I (mouse specific; 100 and 130 bp) and *Fok*I (human specific; 35 and 200 bp) were used for restriction analysis of the PCR product. For MMP-2, specific primers GGC-CCT-GTC-ACT-CCT-GAG-AT (sense) and GGC-ATC-CAG-GTT-ATC-GGG-GA (antisense; PCR product, 473 bp; 30 cycles; T_{ann} 55°C) were used, and for restriction analysis of the PCR product, *Sca*I (mouse specific; 235 and 240 bp) and *Acc*I (human specific; 184 and 290 bp) were used. For MMP-9, specific primers CAA-CAT-CAC-CTA-TTG-GAT-CC (sense) and CGG-GTG-TAG-AGT-CTC-TCG-CT (antisense; PCR product, 479 bp; 30 cycles; T_{ann} 55°C) were used, and for restriction analysis of the PCR product, *Alu*I (mouse specific; 420 bp) and *Eco*I (human specific; 200 and 280 bp) were used. All restriction enzymes were obtained from Promega.

Nested RT-PCR for VEGFR-2 was performed as follows: (*a*) first PCR was performed using CTT-CAA-CCA-GTC-TGG-GAG-TGA-GA (sense) and CTC-TCC-TGC-TCA-GTG-GGC-TGC-ATG-T (antisense; PCR product, 860 bp; 25 cycles; 1 mM $MgCl_2$ and 5% DMSO; T_{ann} 60°C); and (*b*) nested PCR was performed using TGG-AAG-TGG-CAT-GGA-ATC-TC (sense) and TTG-CCG-CTT-GGA-TAA-CAA-GG (antisense; PCR product, 571 bp; 25 cycles; T_{ann} 53°C) with 4 μl of the first PCR product.

Nested RT-PCR for ANG-2 was performed as follows: (*a*) first PCR was performed using TGA-GCA-AAC-GCG-GAA-GT (sense) and TTC-TTC-TTT-AGC-AAC-AGT-GGG (antisense; PCR product, 423 bp; 30 cycles; 1 mM $MgCl_2$; T_{ann} 62°C); and (*b*) nested PCR was performed using CTT-GGA-ACA-CTC-CCT-CTC-G (sense) and GTC-CTT-AGC-TGA-GTT-TGA-TGT-GG (antisense; PCR product, 330 bp; 30 cycles; 1 mM $MgCl_2$; T_{ann} 60°C) with 4 μl of the first PCR product. Restriction analysis of this PCR product was performed using *Nhe*I (human specific; 106 bp) and *Bst*OI (mouse specific; 169 bp).

To control for the addition of cDNA in the first PCR reaction, β_2-microglobulin PCR was performed from the same first PCR product as used for the second VEGFR-2 PCR (nested PCR). The first product was split; 4 μl were used for the β_2-microglobulin PCR to control for the cDNA addition in the first PCR.

Statistical Analysis. Nonparametric tests such as the Mann-Whitney *U* test and Spearman analyses were used for the abnormally distributed data. $P < 0.05$ was considered to represent significance.

RESULTS

Xenotransplantation Model. After sublethal irradiation of mice followed by s.c. injection of 10×10^6 Daudi cells, measurable tumors developed at the injection site (in the neck region) of all mice (Fig. 1*A*). Tumors grew as solid masses in the neck region. Tumors were composed of CD20+/CD22+ cells, with membrane IgM expression typical for Daudi cells (Fig. 1*B*). No large areas of necrosis were observed (data not shown). When left untreated, the tumors reached a weight of approximately 600 mg at day 24. In this s.c. model, no metastases were found ever during the therapeutic window.

Mouse and Human Angiogenic Factors Are Expressed in the Tumor. The tumors were well vascularized, as shown by staining with CD31 (Fig. 1*B*). No differences in vessel number could be observed between the center and peripheral areas of the tumors (data not shown). Accordingly, we determined the expression of several angiogenic factors including ANG-2, bFGF, MMP-2, MMP-9, and VEGF by RT-PCR. In addition, we assessed whether these factors were of mouse or human origin. *In vitro* cultures of Daudi cells demonstrated the expression of human VEGF and MMP-9 mRNA, but not transcripts for ANG-2, bFGF, and/or MMP-2 (Fig. 2*A*). However, in the tumor sections, not only were VEGF and MMP-9 found (as expected), but mouse-specific ANG-2, MMP-2, and bFGF

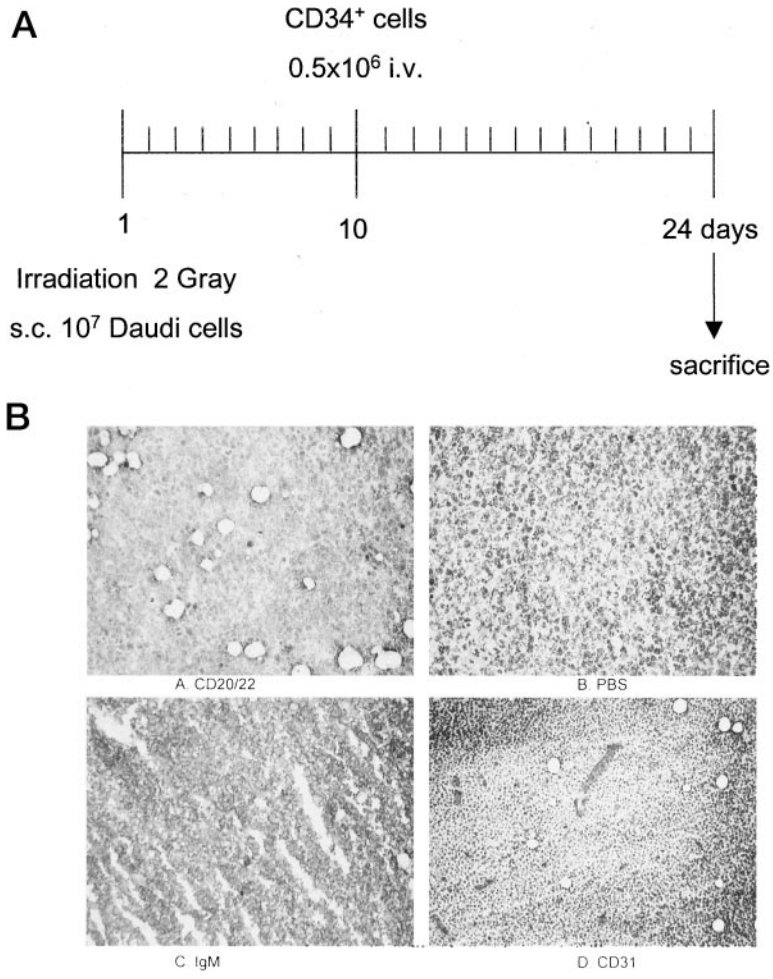


Fig. 1. *A*, the design of the study using a NOD/SCID mouse model of human non-Hodgkin's lymphoma with or without mobilized CD34+ human hematopoietic stem cells i.v. *B*, immunohistochemical staining of Daudi tumor in mice for CD20/CD22, IgM, and CD31. Cells were counterstained with hematoxylin; magnification, ×10.

were found as well, as determined by restriction enzyme analysis (Fig. 2*B*). No differences in expression of the investigated angiogenic factors were observed between the center of the tumors and the peripheral areas (data not shown).

Human CD34+ Hematopoietic Cells Increase Tumor Growth. To determine whether CD34+ hematopoietic cells enhance tumor growth, human CD34+ hematopoietic stem cells obtained after mobilization were injected i.v. at day 10 in this xenotransplantation model at a dose of 0.5×10^6 cells/mouse. At this time point, no palpable tumor was found in the neck region; however, with immunohistochemical staining, a distinct CD20+/CD22+ tumor was found comparable to the clinical situation of minimal disease. The mobilized CD34+ human progenitors were CD34+, CD10−, CD19−, CD7−, CD56−, GPA−, and BB4−. At day 24 after tumor inoculation, the mice were sacrificed. Fig. 3*A* shows that the tumor weight in the xenotransplanted mice infused with human CD34+ cells is nearly 2-fold greater than that seen in xenotransplanted mice infused with control PBS [median CD34+ group (1086 mg; range, 411-1287 mg; $n = 12$) *versus* PBS group (586 mg; range, 270–976 mg; $n = 11$), $P = 0.004$]. When using the CD34− fraction of MNCs from leukapheresis samples, the tumor growth was not enhanced compared with that seen in PBS controls [median CD34− fraction (749 mg; range,

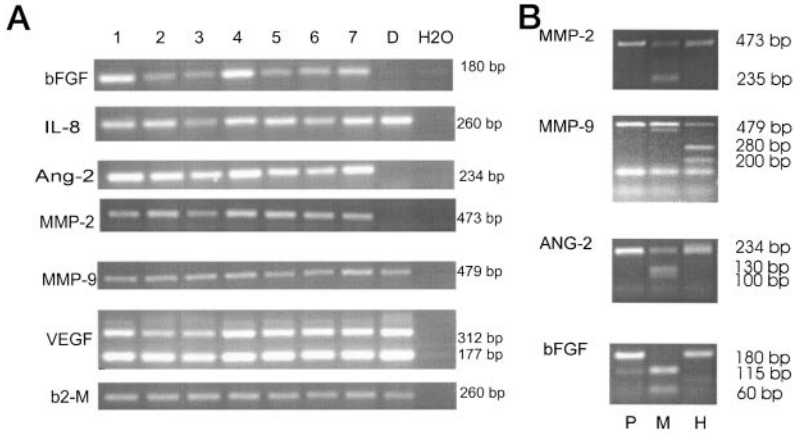
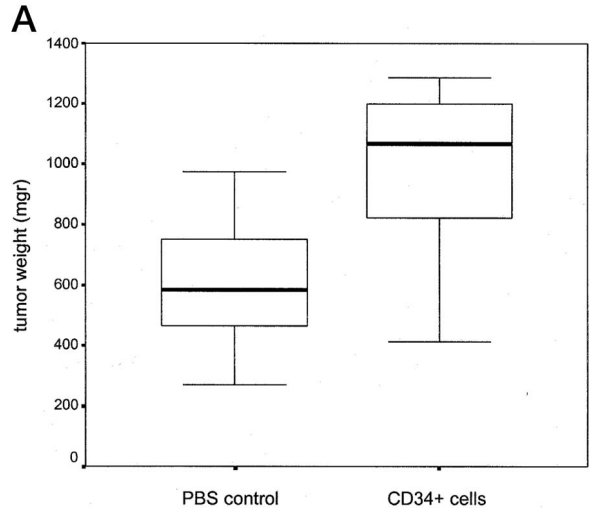


Fig. 2. *A*, the expression of ANG-2, IL-8, bFGF, MMP-2, MMP-9, and VEGF by RT-PCR in Daudi tumor in mice and Daudi cells *in vitro*. *B*, the mouse or human origin of the angiogenic factor was shown by enzyme restriction analysis (see "Materials and Methods"). *Lane P*, PCR product; *Lane M*, enzyme restriction with mouse specific enzyme; *Lane H*, enzyme restriction with human specific enzyme.



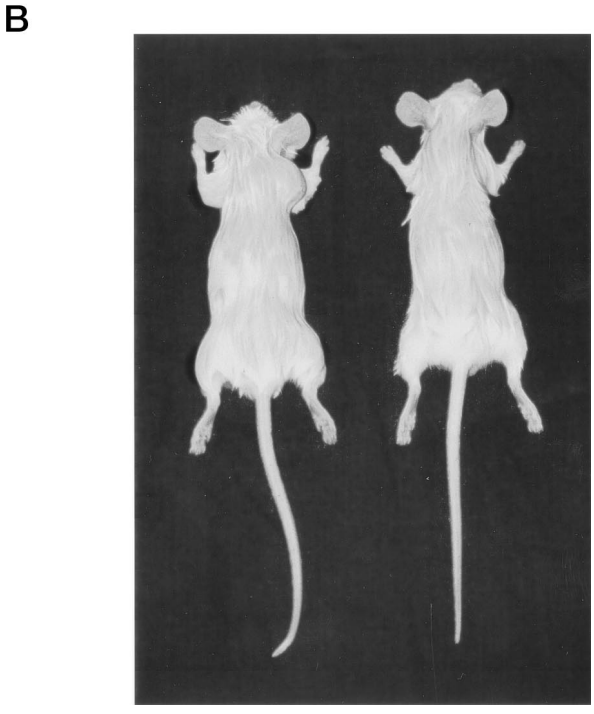


Fig. 3. *A*, growth of Daudi tumor in a NOD/SCID model with or without the addition of CD34+ human hematopoietic stem cells; the *left bar* represents the median in box plot (25–75 percentiles) of tumors in PBS control mice, and the *right bar* represents tumors in mice injected with CD34+ cells ($P = 0.004$). *B*, difference in tumor growth in two representative mice. *Right mouse*, PBS control mouse; *left mouse*, mouse injected with CD34+ cells.

685–858 mg; $n = 3$) *versus* median PBS controls (586 mg; range, 270–976 mg; $n = 11$). In addition to 0.5×10^6 CD34+ cells, 0.1×10^6 CD34+ cells were injected to show a relation between the number of injected CD34+ cells and the increased tumor growth. A 5-fold lower amount of CD34+ cells resulted in a tumor of 443 mgr (range, 424–462 mgr; $n = 2$). This suggests that a certain number of injected CD34+ cells are needed to increase the size of the tumors. In Fig. 3*B*, the difference in tumor growth is visualized for two representative animals. No differences could be found with regard to morphology, IgM, CD20/CD22, CD31, and FVIII stainings between tumors from mice infused with CD34+ cells and tumors from mice injected with PBS. No differences were found in the number of vessels/high-power field; however, the total tumor mass was increased by 2-fold.

Mice injected with human CD34+ cells alone demonstrated no outgrowth of myeloma cells after a follow-up time of 2 months, as measured by PCR of myeloma-specific V(D)J transcripts based on the hypervariable CDR1 and CDR3 regions of the immunoglobulin rearrangements expressed by myeloma cells. However, in bone marrow and spleen, human cells expressing CD19 and CD38 were found in numbers varying between 0.4% and 19.6% (in bone marrow) and 0.7% and 6.1% (in spleen; $n = 8$). This indicates that the NOD/SCID bone marrow and spleen microenvironment supports early human B lymphopoiesis.

Human CD34+ Hematopoietic Stem Cells Can Be Found in the Tumors. To demonstrate that the injected human CD34+ cells have been homed to the tumors and differentiated along the endothelial lineage to (precursor) endothelial cells, RT-PCR studies were performed with oligonucleotides specific for human VEGFR-2, which is expressed on CD34+ cells. As depicted in Fig. 4*A*, VEGFR-2 mRNA

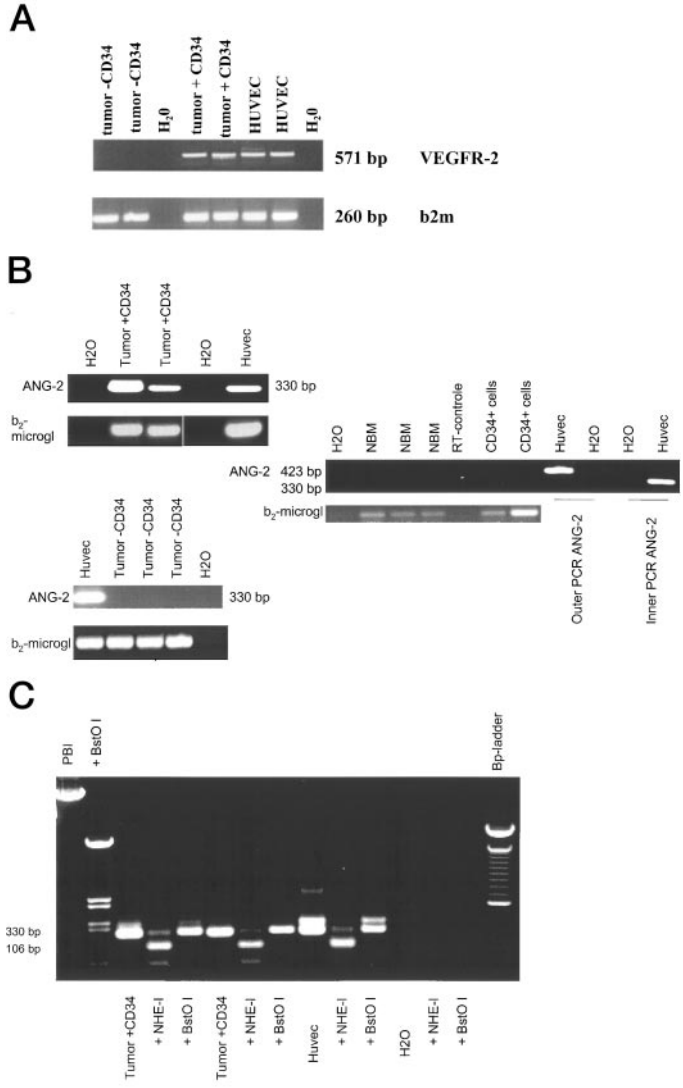


Fig. 4. *A*, expression of human-specific VEGFR-2 in Daudi tumors from mice with CD34+ cells i.v. *versus* control mice. A nested RT-PCR for VEGFR-2 is used as described in "Materials and Methods." As a control for the addition of cDNA in the first PCR reaction, the expression of β_2-microglobulin by RT-PCR is shown for the different samples. *B*, expression of human-specific ANG-2 in Daudi tumors from mice with CD34+ cells i.v. *versus* control mice. A nested PCR is used as described in "Materials and Methods." CD34+ cells, bone marrow MNCs (*NBM*), and umbilical endothelial cells (*Huvec*) are used as controls. *C*, ANG-2 PCR bands are human and not murine, as shown by enzyme restriction analysis. *NheI* is the enzyme recognizing human ANG-2, and *BstOI* recognizes mouse ANG-2. Control for *BstOI* is shown in the *first two lanes*.

is expressed by the tumors from mice injected with CD34+ cells, whereas in the control mice, no transcript was shown. In all samples, β_2-microglobulin PCR was performed with the same first PCR product to confirm the equivalent addition of cDNA.

Moreover, human-specific RT-PCR for ANG-2 was done to ensure that these human CD34+ cells differentiate along the endothelial lineage. Fig. 4*B* shows the results; ANG-2 mRNA (human specific) is expressed by tumors from mice injected i.v. with CD34+ cells, whereas in control mice, no ANG-2 transcript was found. In addition, CD34+ cells and Daudi cells in culture are ANG-2−. With enzyme restriction analysis (Fig. 4*C*), it was demonstrated that the ANG-2 PCR bands were of human origin and not of murine origin.

DISCUSSION

In the present study, it is demonstrated that CD34+ hematopoietic cells injected i.v. might facilitate the outgrowth of tumors in the setting of minimal residual disease. The tumor growth is enhanced 2-fold when mobilized CD34+ hematopoietic cells are given i.v. 10 days after s.c. injection of Daudi cells. The relation of tumor outgrowth and infusion of CD34+ cells is further underscored by the fact that human VEGFR-2 and ANG-2 transcripts could only be found in the tumors of mice treated with CD34+ cells.

This xenotransplantation model was chosen because it has the advantage of 100% engraftment, and spontaneous regression of tumors is rarely seen (27). As shown by Hudson *et al.* (27), optimal growth of Daudi cells is achieved using NOD/SCID mice in conjunction with sublethal radiation. The disadvantage of this model is the ectopic localization of the tumor outside its normal environment. However, the actual biology or related immune function was not the aim of this study.

Tumor-induced blood vessel formation is the result of a strong interaction between tumor cells and surrounding normal cells, such as endothelial cells or their precursors. In the present study, this is reflected by the production of VEGF and MMP-9 of human origin and MMP-2, bFGF, and ANG-2 of murine origin; it is likely that all these factors contribute to tumor growth because murine MMP-2 and bFGF affect human cells.

Recent data have indicated that a circulating common precursor for hematopoietic and endothelial cells might exist that is phenotypically characterized by the expression of VEGFR-2 (12–15). The existence of a bipotential precursor (hemangioblast) for hematopoietic and endothelial cells was suggested by the finding that embryos lacking VEGFR-2 demonstrated defects in both hematopoietic and endothelial lineages (28, 29). In normal homeostasis, a low amount of mature endothelial cells (0.05%) can be detected in the peripheral circulation (30–32). It is believed that these circulating endothelial cells are mainly the result of turnover from vessel wall endothelium and can be expanded slowly in *in vitro* cultures (33). In contrast, bone marrow-derived endothelial cells demonstrate a 50-fold faster expansion but are even 20-fold more sparse in the peripheral blood. However, during the mobilization of bone marrow hematopoietic precursors, the number of circulating progenitors in the peripheral blood is increased from 0.05% or less up to 1–5%. This increase is comparable to the effect of i.v. infusion of human CD34+ cells in this mice tumor model. It is not known whether these mobilized circulating endothelial cells are involved in enhanced outgrowth of tumors in humans. However, in human chronic myeloid leukemia, it was recently shown that the BCR/ABL fusion transcript was expressed in a variable proportion of endothelial cells (34).

Endothelial precursors originating from hematopoietic stem cells remain VEGFR-2+ and might become ANG-2+ during their differentiation, whereas hemangioblasts committed to the hematopoietic lineage showed no Ang-2 transcripts and lose their VEGFR-2 expression. Our results strongly suggest that injected CD34+ human cells from peripheral blood stem cell sources can be found in the tumor as human VEGFR-2+ and ANG-2+ cells. The possibility that outgrowth of circulating endothelial cells from the peripheral blood enhance tumor growth by new vessel formation cannot be excluded. However, the number and proliferating potential of circulating endothelial cells are far less then those of bone marrow-derived CD34+ cells, as discussed above. Dedifferentiation of Daudi cells to precursor endothelial cells cannot be excluded in this model. However, Daudi cells are CD34−, VEGFR-2−, and ANG-2−, and dedifferentiation of Daudi cells has not been described until now.

These results show for the first time that malignant tumors are capable of incorporating mobilized CD34+ hematopoietic cells, resulting in enhanced tumor growth. The study suggests that leukapheresis and resubmission of mobilized stem cells in patients with residual tumor might lead to enhanced tumor growth and must be done with caution. Further investigations are warranted to ensure that leukapheresis and/or stem cell reinfusion in the setting of autologous stem cell transplantation in patients with residual disease are a safe method of treatment.

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