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Research Article

Long -Term Activation of Circulating Liver-Committed Mononuclear Cells after OLT

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Abstract

Liver viability after orthotopic liver transplantation (OLT) is reportedly dependent on extrahepatic stem cells of host bone marrow origin. Liver-specific proteins were detected in the blood 2 weeks after OLT. We hypothesized that OLT induces long-duration recruitment of such cells committed to the graft tissue. Two fractions of cells related to angiogenesis (CD133+ and CD31+) were analyzed by flow cytometry in mononuclear blood cell (MNC) samples from 8 recipients to quantify the alpha-fetoprotein (AFP)-positive subsets recruited into circulation 2.5–60 months after OLT. The blood of 6 healthy volunteers and 7 patients with cured lung carcinoma (LC) were used as controls to minimize the nonspecific effects of surgery and chemotherapy. Mean numbers of CD133+AFP+ mononuclear cells and CD31+AFP+ lymphocytes increased several times after OLT and remained enhanced 5 years after transplantation compared with those in control subjects. These results show induction and navigation of angiogenic cells for graft repair during long-term adaptation to the host. The liver graft can be a long-term stimulus for recruitment of additional CD133+ stem cells and CD31+ lymphocytes with the liver-specific protein AFP into the peripheral blood, and then to the vascular endothelium and hepatoblasts, which are the most probable targets for AFP+ migrants.

Keywords: Hematopoietic Stem Cells (HSC); Blood; Orthotopic Liver Transplantation (OLT); Alpha-Fetoprotein (AFP)-Positive Subsets; Long-Term Enhancement

Introduction

Liver transplantation is the only definitive treatment for the end-stage liver disease. The cellular mechanisms that govern normal liver regeneration are not fully understood. Bone marrow (BM) is reportedly one of the main external systems providing for organs, including the liver. The hepatocyte mass is kept stable through a tight balance between cell death and proliferation, which is frequently lost following acute or chronic liver pathology. Strongly proliferating multipotent CD133+ stem cells of BM origin differentiate into cells with characteristics of mesoderm, endoderm, and neuroderm layers, such as endothelial progenitor cells, neural progenitor-like cells, astrocytes, oligodendrocytes, kidney proximal tubule cells, lactiferous duct cells of the mammary gland, prostate gland cells, skin cells, lung cells, intestinal cells, skeletal muscle-like cells, and hepatocyte-like cells [1,2].

Only a minority of hematopoietic stem cells (HSCs) in humans differentiates into liver lineages, and the majority fuse with resident hepatocytes, as shown by X-Y chromosomes following cross-sex BM transplantation. Thus, the observed improvements in liver function due to transplanted BM

cells or BM-derived HSCs are attributed to their secretion of growth factors and cytokines, rather than transdifferentiation [3-5]. The concept of a paracrine proliferative effect of HSCs on hepatocytes has been supported by the beneficial effect of repeated infusion of HSCs after gradual worsening of an initial improvement in liver function in patients with end-stage liver disease [6]. Although CD133+ HSCs are also related to angiogenesis, they have been reported as cancer stem cells in hepatocellular carcinoma (HCC). The specificity of CD133 as an HCC marker seems ambiguous because CD133 provides only common stem cell properties, including higher proliferative potential, self-renewal, and differentiating capacity, as markers, and it is an effective prognostic factor for overall survival only in patients with stage I HCC [7]. Cancer can develop from CD133-negative cells [8]. Moreover, at least, some normal hepatic oval progenitor cells are derived directly from precursors of BM origin, as they contain several antigens traditionally associated with hematopoietic cells and are under paracrine control [9]. Spontaneous migration of primitive 133+ and 34+ cells from BM through the blood into different tissues and organs, particularly after injury, induces regenerative processes in most normal organs [10], as well as in malignant tissues [4, 11, 12]. Orthotopic liver transplantation induces recruitment of pluripotent BM-derived CD133+ HSCs, CD34+ HSCs, double-positive CD133+ CD34+ cells, terminal deoxynucleotidyl transferase (TdT)+ prelymphocytes, vascular endothelial growth factor (VEGF)+ mononuclear cells (MNCs), CD31+ MNCs , and some Treg subpopulations into peripheral blood 1 month after surgery. Such a generalized shift of hematopoiesis toward young subsets and the concomitant reduction of the majority of more mature subpopulations in host blood are the result of graft implantation and anti–graft rejection cytotoxic treatment [13, 14]. Migration of HSCs through the blood, across the endothelial vasculature into the graft, requires active navigation called homing. Homing has physiological roles in homeostasis, during which cells from the BM reservoir are recruited and home toward a specific organ for repair [15]. Transcripts of liver-specific epithelial markers, such as cytokeratin 19 and alpha-fetoprotein (AFP), were discovered in total cell RNA extracted from peripheral blood MNCs of two patients 7 days after OLT, coinciding with an increase in the number of circulating CD34+ HSCs [13]. However, no data describe the number of circulating cells possessing specific attractiveness to transplanted liver tissues or the duration of this attraction. However, such data are important to develop novel strategies for the treatment of patients undergoing liver transplantation.

The goals of this study were to evaluate and quantify the number of AFP-positive tissue-committed cells at two stages of differentiation in the blood of patients who have undergone OLT, to detect the cells over the long term by flow cytometry, and to determine whether long-term quantitative changes in these cells differ from those in a control group.

Materials and Methods

This study was conducted at the Federal Scientific Centre for Radiology and Surgical Technologies, Saint-Petersburg, Russia, which is a comprehensive cancer center of the Ministry of Health Care, Russia. We investigated the peripheral blood of 21 adult subjects after obtaining written informed consent from each of them.

Patients

The working group was randomized one, consisting of 21 subjects. The age of the subjects ranged from 40 to 58 years, the sex ratio was 14 female and 7 males.

Group 1 consisted of eight adults (average age -51 years, sex ratio -5 female and 2 males) with liver cirrhosis (four viral, four autoimmune) undergoing OLT and treated with basiliximab and immunosuppressive therapy, including tacrolimus (n = 7 of 8) and mycophenolate with prednisone (n = 8 of 8). Group 2 comprised seven adults (average age -49 years, sex ratio -5 female and 3 males) with advanced non-small LC who received conventional chemo-radiotherapy after palliative surgery. Patients with LC were chosen because of the high probability (41%) of liver affection. Group 3 consisted of six healthy adult volunteers (average age -55 years, sex ratio -4 female and 2 males).

Blood samples were taken before transplantation (group 1) or treatment (group 2).

Then, blood from each treated patient in groups 1 and 2 was taken repeatedly at a convenient time within 2,5-60 months: 2-3, 7-9, 13-15, 20-23, 30-38, 44-46, 54-56 and 57-62 months after the start of treatment.

Flow cytometry analysis

MNCs were isolated by classical Ficoll density separation from 10 ml peripheral blood. An LSR Fortessa flow cytometer (Becton-Dickinson, San Jose, CA, USA) was used for immunofluorescent detection of CD133+ and CD31+ AFP+ cells in MNCs using the BD Cytometer Setup and Tracking Beads Kit. The Lph and Mn fractions were separated by gating on forwarding (FSC) and side (SSC) scatter dot plots, excluding cellular debris. The phenotype of the circulating cells was evaluated by conventional dual color immunofluorescence using a panel of allophycocyanin (APC)-conjugated anti-CD133/2 monoclonal antibodies (MoAbs), fluorescein isothiocyanate (FITC)-conjugated anti-CD31 MoAbs, and phycoerythrin (PE)-conjugated anti-AFP MoAbs.

The APC-conjugated MoAbs were purchased from Miltenyi Biotec (Bergisch Gladbach, Germany), the FITC-conjugated MoAbs were from BD Bioscience Pharmingen (San Jose, CA,

USA), and the PE-conjugated MoAbs were from R & D Systems (Minneapolis, MN, USA).

Negative controls were isotype-matched irrelevant MoAbs (Becton-Dickinson).

The analysis was performed immediately after the samples were taken from patients, around 11:00 a.m. A minimum of 500,000 total events was recorded twice for assurance when the AFP+133+ cells were investigated in the Lph fraction. The percentage of positive cells was calculated by subtracting the value of the appropriate isotype control.

The numbers of AFP-positive cells were calculated as follows: proportion (%) of positive AFP+133+ subset in the Lph pool; proportion (%) of the positive AFP+31+ subset in the Mn pool; proportion (%) of AFP+CD133+ subset in the CD133+ fraction of Lph pool.; proportion (%) of AFP+CD31+ subsets in the CD31+ fraction of Mn pool.

Statistical analysis

The data were averaged between 2, 5-60 months in group 1 and in group 2. Then the mean percentages of AFP+ cells were compared with correspondent pre-treatment mean values and mean values in group 3. with Student's *t*-test. A *p*-value ≤ 0.05 was considered to be significant.

Kinetic analysis

Time-related curves were generated for data points in groups 1 and 2 to analyze the main mean values more precisely. Curve fitting was performed with the Microsoft Excel program [32]. We defined the functions that best described the trends in the data using equations. To obtain a line that adequately described the trend with a satisfactory coefficient determination R^2 value, we used higher-order polynomial approximations. R^2 is a statistical measure of the approximation of the data to the fitted regression line. The maximal R^2 value was used to assess the function's goodness of fit to the data. A regression *t*-test was used to confirm the *R* value, using a *p*-value [33]:

$$t = \sqrt{[R^2(n-2)/(1-R^2)]} \quad (1)$$

Results

Long-term increase in circulating liver-committed mononuclear cells after OLT

The healthy volunteers and patients with lung carcinoma (LC) had statistically similar proportions of CD133+AFP+ or CD31+AFP+ cells in lymphocyte (Lph) and monocyte (Mn) pools, as well as similar proportions of these subsets in the corresponding CD133+ and CD31+ fractions of these pools (Table 1).

In contrast, to volunteers, those who were *awaiting* liver transplantation had a smaller proportion of CD133+AFP+ cells in the Lph pool (5.5-fold, $p = 0.05$; Figure 1A) and in the *CD133+ Lph fraction* (3.8-fold, $p = 0.03$; not shown).

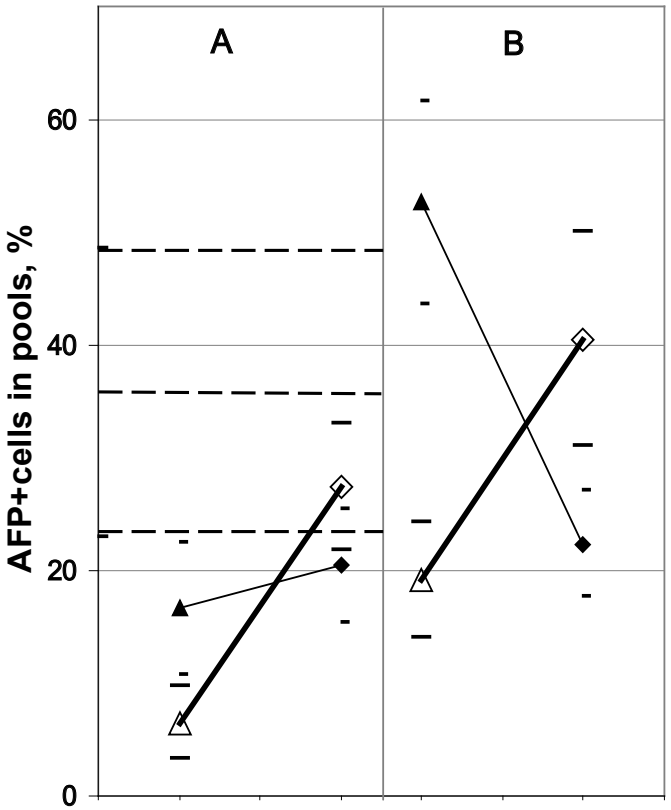

Figure 1. Mean parameters of CD133+ alpha-fetoprotein (AFP)+ circulating cells committed to the liver in patients with liver cirrhosis and patients with lung cancer (LC).

A-in total lymphocyte (Lph) pool, %×10⁴; B-in total monocyte (Mn) pool, %×10². Closed symbols- for lung cancer; open symbols- for liver transplantation. Triangles-before treatment; rhombuses-2,5-60 months after liver transplantation or during lung cancer treatment. Dottedlines- M±SE for volunteers.

After transplantation, the mean proportion of CD133+AFP+ cells in the Lph pool increased compared with that *before* transplantation and returned to the level of the volunteers (4,2-fold, $p = 0.008$; Figure 1A).

In contrast to patients with *untreated* LC, those who were *awaiting* liver transplantation had a smaller proportion of CD133+AFP+ cells in the Mn pool (2.7 fold, $p = 0.01$; Figure 1B). During the several years after transplantation, the proportion of CD133+AFP+ cells in *CD133+ Mn fraction* increased more than two-fold (2.2 fold, p=0.013, Figure 2A).

Cells	AFP+CD133+ cells, % (M± SE)				AFP+CD31+ cells % (M± SE)			
	in pool		in CD133+ fraction		in pool		in CD31+ fraction	
	LC	volunteers	LC	volunteers	LC	volunteers	LC	volunteers
Lph	0.00166±0.00059	0.00357±0.00128	10.84±3.49	18.09±5.13	0.47±0.21	0.100±0.031	1.93±0.86	0.20±0.077
p	n.s.		n.s.		n.s.		n.s.	
Mn	0,00526±0.0090	0.0487±0.0215	27.66±7.37	22.4±7.70	22.46±7.5	24.5±4.11	56.4±6.7	59.3±16.8
p	n.s.		n.s.		n.s.		n.s.	

Table 1. AFP+ mononuclear cells in the blood of the volunteers and patients with lung cancer (LC) *before* treatment.
n.s.- not significant (p>0,05).

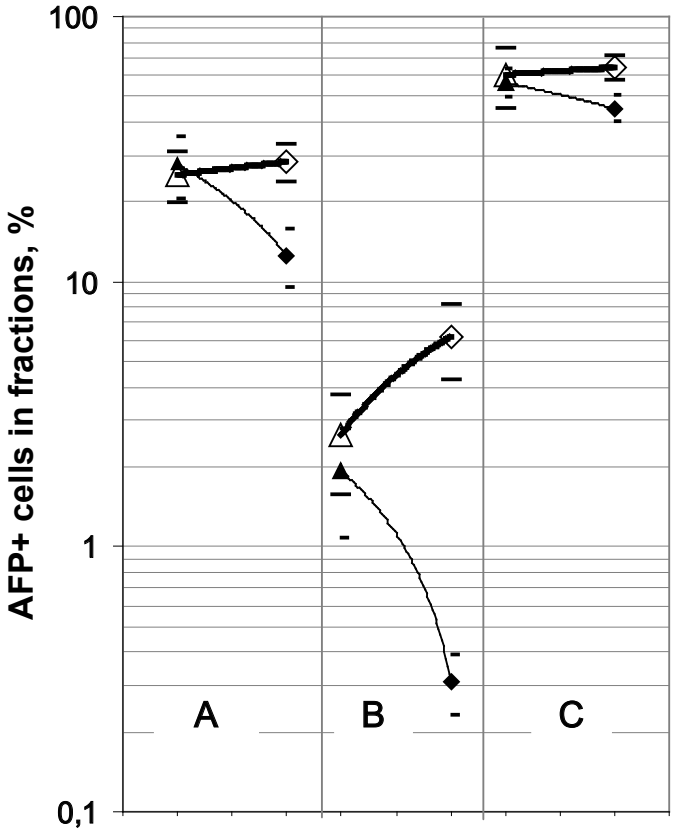

Figure 2. Mean parameters of alpha-fetoprotein (AFP) + circulating cells committed to the liver in patients with liver cirrhosis and patients with lung cancer (LC).

A-in fraction of CD133+ monocytes (Mn), %; B-in fraction of CD31+ lymphocytes (Lph), %; C- in fraction of CD31+monocytes (Mn). Closed symbols- for lung cancer; open symbols- for liver transplantation. Triangles-before treatment; rhombuses-2,5-60 months after treatment.

In parallel, the level of CD31+AFP+ cells in the Lph pool *after* transplantation was higher compared with *treated* LC patients and those who were *awaiting* liver transplantation (9.3-fold, *p*=0,013 and 2.4- fold, *p*=0,011respectively; Figure 3A).

The proportion of CD31+AFP+ cells in the *CD31+ Lph fraction in waiting for patients*, which was comparable with that in the untreated LC patients, increased 20-fold after transplantation (p = 0.011, Figure 2B). Remarkably, CD31+AFP+ cells in the Mn pool were 2.9-fold lower than in volunteers before transplantation (p = 0.013) and remained *unchanged* after transplantation (p = 0.016; Figure 3B). Only the proportion of CD31+AFP+ cells in the CD31+ Mn *fraction* increased slightly compared with that in treated LC patients (1.4-fold, *p*=0.04; Figure 2C).

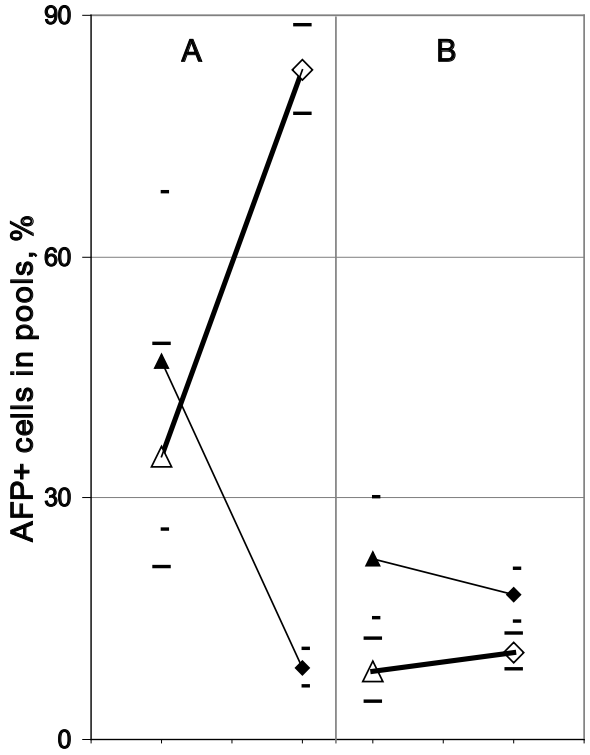

Figure 3. Mean parameters of CD31+ alpha-fetoprotein (AFP)+ circulating cells committed to the liver in patients with liver cirrhosis and patients with lung cancer (LC).

A-in total lymphocyte (Lph) pool, %×10²; B-in total monocyte (Mn) pool, %. Closed symbols- for lung cancer; open symbols- for liver transplantation. Triangles-before treatment; rhombuses-2,5-60 months after liver transplantation or during lung cancer treatment.

The proportion of CD31+AFP+ cells in the *CD31+ Lph fraction in waiting for patients*, which was comparable with that in the untreated LC patients, increased 20-fold after transplantation (p=0.011, Figure 2B). Remarkably, CD31+AFP+ cells in the Mn pool were 2.9-fold lower than in volunteers before transplantation (p=0.013) and remained *unchanged* after transplantation (p=0.016; Figure 3B). Only the proportion of CD31+AFP+ cells in the CD31+ Mn *fraction* increased slightly compared with that in treated LC patients (1.4-fold, p=0.04; Figure 2C).

Time-related curves allow analyzing the mean values in groups 1 and 2 more precisely

Figures 4 and 5 present the results of AFP+ cell monitoring in the blood of patients at different times after they started appropriate treatment.

Figures 4 and 5 show the proportional values during the 60 months after the initiation of cancer therapy or after liver transplantation. Figure 4 (rhombuses) explains why no difference was found in *the mean proportion* of CD133+AFP+ cells in the CD133+ Lph fraction between patients who underwent OLT and patients with LC after treatment (6.15±1.39 % and 7.35± 2.67 % respectively, not shown in Figure 2). The proportion of CD133+AFP+ cells in the 133+Lph fraction was *higher* 2.5–20 months after liver transplantation, whereas it became *lower* after 35–50 months compared with that at curing LC (Figure 4).

All we could do was find the model that best describes the data Figures 4 and 5. The p-values in Table 2 are simply a measure of how the well model of *fluctuations* fits the data and not a criterion of significance.

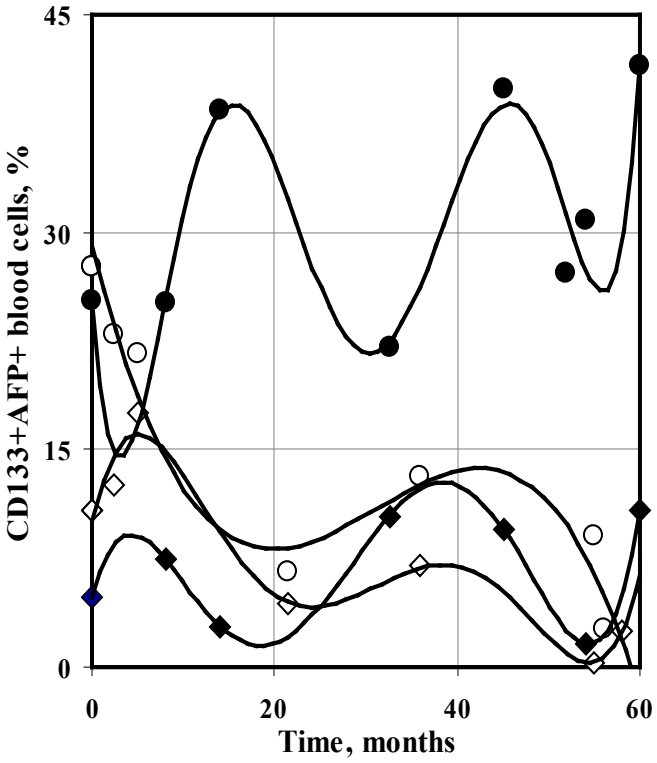

Figure 4. Proportions of CD133+ alpha-fetoprotein (AFP)+ cells in the CD133+fractions of the total lymphocyte (Lph) and monocyte (Mn) pools after liver transplantation or during lung cancer (LC) treatment. Rhombuses - Lph; circles - Mn; closed symbols - transplantation; open symbols - LC.

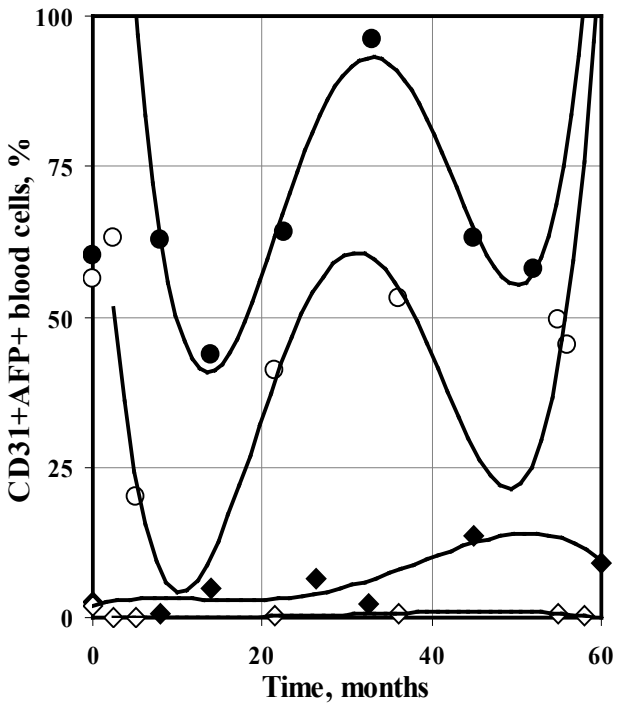

Figure 5. Proportions of CD31+ alpha-fetoprotein (AFP) + cells in the CD31+ fractions of total lymphocyte (Lph) and monocyte (Mn) pools after liver transplantation or during lung cancer (LC) treatment. Rhombuses - Lph; circles - Mn; closed symbols - transplantation; open symbols- LC.

Cells, Figures	Liver transplantation, Equation, R^2, p	Lung cancer, Equation, R^2, p
AFP+in 133+ Lph (Fig. 4)	$y = 3E\text{-}06x_5 - 0.0004x_4 + 0.0198x_3 - 0.382x_2 + 2.3133x + 4.767$ $R^2 = 1$, $p < 0.001$	$y = 0x_5 - 0x4 + 0x_3 - 0x_2 + 3x + 10$ $R^2 = 1$, $p < 0.001$
AFP+in 133+ Mn (Fig. 4)	$y = 4E\text{-}07x_6 - 6E\text{-}05x_5 + 0.0043x_4 - 0.1338x_3 + 1.824x_2 - 7.9865x + 25.267$ $R^2 = 0.930$, $p < 0.001$	$y = -0.001x_3 + 0.092x_2 - 2.4923x + 29.044$ $R^2 = 0.951$, $p < 0.001$
AFP+in 31+ Lph (Fig. 5)	$y = -0.0002x_3 + 0.0203x_2 - 0.2801x + 2,8443$ $R^2 = 0.628$, $p = 0.03$	$y = -2E\text{-}06x_4 + 0.0001x_3 - 0.0029x_2 + 0.0171x + 0.1147$ $R^2 = 0.911$, $p = 0.003$
AFP+in 31+ Mn (Fig. 5)	$y = 0.0004x_4 - 0,0517x_3 + 2.227x_2 - 35,871x + 232,94$ $R^2 = 0.976$, $p < 0.001$	$y = 0.0003x_4 - 0.0403x_3 + 1.5764x_2 - 21.146x + 94.992$ $R^2 = 0.679$, $p = 0.04$

Table 2. Kinetic and statistical parameters used to approximate lines in Figures 4 and 5. *

Fluctuations in the proportions of AFP+ cells might be real as demonstrated by the high *R* values for non-linear equations (Table 2) and by the similarity between pairs of lines (liver graft and LC) for CD133+AFP+Lph (Figure 4) and CD31+AFP+Mn (Figure 5). Thus, all mean parameters (M±SE) in Figures 1, 2 and 3 were received after treatment at the turbulent state of myelopoiesis.

Discussion

AFP has only been described qualitatively by RT-PCR as a specific liver marker in human blood after OLT in CD34+ HSCs on day 7 after surgery [13]. However, the primitive blood MNCs in adults are represented by CD133+CD34−, CD133+CD34+, and CD133−CD34+ markers, and the CD133 marker is ancestral to CD34 [14]. We examined the younger CD133+cells compared with the CD34+ hematopoietic progenitors. Among the many properties of the circulating pluripotent CD133+ cell subset is its reliable ability to support regeneration of hepatocytes and angiogenesis [16-18]. The vasculogenic potency of circulating blood also includes more mature CD31+ angiogenic T-lymphocyte [19]. These blood cell properties and their homing ability to different tissues complicate the understanding of allograft viability and rejection; for this reason, we included the CD133+ and CD31+ subsets in this study. We hypothesized that interactions on the "BM–liver graft" axis are long-term and involve homing of feeding cells as an essential condition of the extrahepatic system for long-term organ regeneration and natural renewal. If this hypothesis is correct, it may affect the practice of quantitative therapeutic HSC injection with unknown specificity to the target tissue.

Increases in the proportions of CD34+ and CD133+ circulating HSCs during the 0.5–1 month after OLT were described recently [13, 14, 20]. In the present study, we monitored the 2.5–60-month post-transplant period to determine whether committed CD133+AFP+ and CD31+AFP+ cells are mobilized / recruited over the long term. Such long-term analysis of angiogenic subsets in the blood of recipients was relatively independent of nonspecific surgical factors, which appear immediately or shortly after surgery. LC was chosen for comparison with transplantation because patients with LC have the highest probability of liver effects [21]. LC is treated with surgery, and patients use myelosuppressive drugs, similar to transplant recipients. These similarities ensured that the LC control was more reliable and strong compared with healthy volunteers.

Nevertheless, we detected no statistical difference in AFP+ cells between the donor groups and patients with LC (Table 1), suggesting the absence of any specific stimulus changing normal homeostasis of liver-committed AFP+ cells in patients with LC and healthy volunteers.

In contrast, we detected the lower percentage of CD133+AFP+ cells in the Lph and Mn *pools* in patients waiting for OLT compared with control patients (5.5-fold, $p = 0.05$ and 2.7-fold, p=0,01**,** respectively; Figure 1 A). The proportions of CD133+AFP+ cell in the Lph *pool* returned to the levels of the healthy controls 2.5–60 months after OLT (Figure 1A).

The levels CD31+AFP+ cells in the Lph *pool* and in CD 31+

fraction before OLT were comparable with corresponding values in *untreated* LC patients (Figures 3A and 2A). A 9-fold increase in the mean number of CD31+AFP+ cells in the Lph *pool* (p = 0.013; Figure 3A) and a 20-fold increase in the proportion of CD31+AFP+ cells in the CD31+Lph fraction (p = 0.011; Figure 2A) relative to treated LC controls, respectively, occurred after OLT.

The percentage of CD31+AFP+ cells in the Mn *pool* was lower than in control before OLT but *did not change* after OLT compared with volunteers or patients with treated LC (Figure 3B).

Thus, the CD133+AFP+ cells in the Lph pools together with the CD31+AFP+ Lph cells reacted more specifically with the grafted tissue than with the physiological stress associated with surgery or subsequent long-term cytotoxic chemotherapy, indicating that transplant, per se, is a strong and specific stimulus for long-term mobilization of AFP+ liver-committed cells in the blood. As the proportion of CD31+AFP+ cells in the Lph pool was 10-fold more than that of young CD133+AFP+ cells, the increase in angiogenic CD31+AFP+ Lph cells seemed to be a logical consequence of long-term amplification of the corresponding CD133+AFP+ progenitors after OLT. These data suggest the imperfect nature of long-term grafts, as extrahepatic cells originating from BM appear when target organ injury is significant [13, 22, 23].

Homing may be related more to hepatocytes than to cholangiocytes because only resident progenitor cells of the hepatocyte lineage are AFP positive [7]. The youngest resident hepatic *stem cells* (HepSCs) are not a target of AFP+CD133+ migrant cells. The phenotypic profile of HepSCs includes CD133 molecules, but not AFP or any marker for hematopoietic (CD34 and CD45) or endothelial (VEGFr and CD31) tissues [24]. In contrast, immature unipotent *hepatoblasts,* which expand during regenerative processes, have an antigenic profile that overlaps partially with that of HepSPCs, and a strongly positive expression of a hepatic-specific AFP marker [25, 26]. They are very likely to target candidates of CD133+AFP+-committed migrants, which are aimed at specific proteins for regeneration in the target tissue.

AFP, which is delivered by CD133+AFP+ and/or CD31+AFP+ cells, may also stimulate angiogenesis in target tissues [7, 26]. Silencing of AFP expression significantly reduces hepatic VEGF expression [27]. Thus, the enhanced proportion of AFP+ cells among the numerous mononuclear CD31+ cell populations (Figures 3A and 2B) may be a long-term reaction allowing the vascular system to adapt to the transplant.

Timely blood samples were difficult to obtain from a few patients in the present study. The variability in the findings after treatment (Figures 4 and 5) may be explained by individual patient characteristics. In addition, the slow hematopoietic changes induced by periodic cytotoxic chemical treatments during the long postoperative period cannot be excluded. The similarity in the statistically optimized curves for patients with liver and lung diseases is another possibility. The curves for CD133+AFP+ Lph in Figure 4 (rhombuses) and CD31+AFP+ Mn in Figure 5 (circles) show synchronous fluctuations in two groups with different diseases. We previously reported the association between the probability of death among patients with advanced cancers and changes in blood CD34+ HSC content, assuming that death is caused by exhaustion of the morphogenic potency of young BM cells [28, 29]. The fluctuations depicted in Figures 4 and 5 may be regular. The similarities in Figures 4 and 5 reflect the long cycles of hematopoietic activity, which may last at least 1 year after injury [30]. Generally, hematopoietic instability is a sign of overstrain by suppressors, which may be harmful to patients compromised by long-term treatment [28, 31].

Conclusion

Flow cytometry of peripheral blood mononuclear cells from eight recipients after orthotopic liver transplantation showed several years of enhanced graft commit and angiogenic AFP+CD133+ and AFP+CD31+ subsets, compared with those of six healthy control volunteers and seven patients with lung carcinoma treated by surgery and cytotoxic chemotherapy. Our results showed that OLT mobilizes additional CD133+ stem cells and CD31+ lymphocytes from BM into the peripheral blood with the liver-specific protein AFP committed to the graft. A transplant can be a long-term stimulus for recruitment of such morphogenic cells during the years following transplantation. The most probable targets for AFP+ migrants are the vascular endothelium and hepatoblasts.

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References

1. Kucia M, Ratajczak J, Ratajczak MZ. Bone marrow as a source of circulating CXCR4+tissue- committed stem cells. In Biol Cell. 2005, 97(2): 133-146.

2. Drapeau Ch. Cracking the stem cell code: demystifying the most dramatic scientific breakthrough of our times. Goodwill Books, Hillsboro, Or, USA Sutton Hart Press; 2010.

3. Schmelzer E. Hepatic progenitors of the liver and extra-Hepatic tissues. In: Liver Regeneration. Edited by Baptista PM. InTech, Science, Technology and Medicine open access publisher. 2012, 43-62.

4. Afroze SH, Jensen K, Rahal K, Meng F, Alpini G et al. Liver

regeneration: stem cell approach. In: Regenerative medicine applications in organ transplantation. 1st. edition .Edited by Orlando G, Lerut J, Soker Sh, Stratta RJ. London: Elsevier, Academic Press; 2014: 375-390.

5. Facciorusso A, Antonino M, Del Prete1 V, Neve1 V, Scavo M P et al. Are hematopoietic stem cells involved in hepatocarcinogenesis? In Hepatobiliary Surg Nutr. 2014, 3(4): 199-206.

6. Zekri AR, Salama H, Medhat E, Musa S, Abdel-Haleem H et al. The impact of repeated autologous infusion of hematopoietic stem cells in patients with liver insufficiency. In Stem Cell Res Ther. 2015, 11(6): 118.

7. Romano M, Francesco FD, Pirozzi G, Gringeri E, Boetto R et al. Expression of cancer stem cell biomarkers as a tool for a correct therapeutic approach to hepatocellular carcinoma. In Oncoscience. 2015, 2(5): 443-456

8. Shmelkov SV, Butler JM, Hooper AT, Hormigo A, Kushner J et al. CD133 expression is not restricted to stem cells, and both CD133+ and CD133–metastatic colon cancer cells initiate tumors. In J Clin Invest. 2008, 118(6): 2111–2120.

9. Roskams T. Controversies in pathology. In: Clinical dilemmas in primary liver cancer. 1st edition. Edited by Williams R, Taylor-Robinson S D, Oxford, UK: Wiley –Blackwell; 2012: 42-52.

10. Stroo I, Stokman G, Teske JD, Florquin S, Leemans J C. Haematopoietic stem cell migration to the ischemic damaged kidney is not altered by manipulating the SDF-1/CXCR4-axis. In Nephrol Dial Transplant. 2009, 24(7): 2082–2088.

11. Shoutko A, Akushevich I, Ekimova L, Karamullin M, Yashin A. The terminal exhaustion of hematopoietic potentiality as the universal cause of death, 38th Annual Meeting of the European Radiation Research Society. September from 5 to 9 2010. Stockholm University, Sweden; 2010.

12. Shoutko AN, Ekimova LP. Lymphocytopenia can contribute in common benefit of cytotoxic therapy of cancer. In Inter Medical. 2014, 3: 5-13.

13. Lemoli RM, Catani L, Talarico S, Loggi E, Gramenzi A et al. Mobilization of done marrow-derived hematopoietic and endothelial stem cells after orthotopic liver transplantation and liver resection. In Stem Cells. 2006, 24(12): 2817-2825.

14. Shoutko AN, Gerasimova OA, Ekimova LP, Zherebtsov FK, Granov AM. Features of subpopulation composition of blood lymphocytes in recipients within the first month after liver transplantation. In Vestnik transplantologii, I iskustbennich organ (Russian journal of transplantology and artificial organs). 2015, 17(1): 68-73.

15. Lapidot T, Dar A, Kollet O. How do stem cells find their way home? In Blood. 2005, 106(6): 1901–1910.

16. Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C et al. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathophysiological neovascularisation. In Circ Res. 1999, 85(3): 221–238.

17. Piscaglia AC, Campanale M, Gasbarrini A, Gasbarrini G. Stem cell-based therapies for liver diseases: state of the art and new perspectives. In Stem Cells International. 2010, Article ID 25946 (10 pages).

18. Conigliaro A, Brenner DA, Kisseleva T. Hepatic progenitors for liver disease: current position. In Stem Cells and Cloning. 2010, **3**: 39–47.

19. Hur J, Yang HM, Yoon CH, Lee CS, Park KV et al. Identification of a novel role of T-cells in postnatal vasculogenesis. In Circulation. 2007, 116(15): 1671–1682.

20. Shoutko AN, Gerasimova OA, Yekimova LP, Granov AM. Fractions of hematopoietic stem cells in a blood of recipients before transplantation of a liver and after it. In Medicinskiy akademicheskiy Zhurnal (Medical Academic Journal) 2013**,** 13**:** 66-70.

21. Nichols L, Saunders R, Knollmann FD. Causes of death of patients with lung cancer. In Arch Pathol Lab Med. 2012, 136(12):1552-1557.

22. Eckersley Maslin MA, Warner FJ, Grzelak CA, McCaughan GW, Shackel NA. Bone marrow stem cells and the liver: are they relevant? In Gastroenterology and Hepatology. 2009, 24(10): 1608–1616.

23. Tonkin J. Establishing a relationship between bone marrow cells and liver progenitor cells by expression profiling and cell tracing studies. Ph.D. thesis, University of Western Australia, Biochemistry and Molecular Biology Department. 2011.

24. Turner R, Lozoya O, Wang Y, Cardinale V, Gaudio E et al. Human hepatic stem cell and maturation liver lineage biology. In Hepatology. 2011, 53(3): 1035-1045.

25. Haruna Y, Saito K, Spaulding S, Nalesnik MA, Gerber MA. Identification of bipotential progenitor cells in human liver development. In Hepatology. 1996, 23(3): 476-481.

26. Liang OD, Korff T, Eckhardt J, Rifaat J, Baal N et al. Oncodevelopmental alpha-fetoprotein acts as a selective proangiogenic factor on the endothelial cell from the

fetomaternal unit. In J Clin Endocrinol Metab. 2004, 89(3): 1415-1422.

27. Meng W, Li X, Bai Z, Li Y, Yuan J et al. Silencing Alpha-Fetoprotein Inhibits VEGF and MMP-2/9 production in human hepatocellular Carcinoma cell. In PLoS ONE. 2014, 9(2): e90660.

28. Shoutko A, Ekimova L, Mus V, Sokurenko V. Fluctuations of CD34+cells number in the blood of cancer patients during a final year of life. In Medical and Health Science Journal, MHSJ. 2012, 13: 7-13.

29. Shoutko AN, Ekimova LP. The impact of middle age on the viability of patients with nonmalignant and malignant diseases. In Cancer Research Journal. 2014**,** 2(6): 114-120.

30. Nothdurft W. Bone Marrow. In: Radiopathology of organs and tissues*.* Edited by Scherer E, Streffer C, Trott K. Berlin, Germany: Springer-Verlag. 1991, 113-169.

31. Fliedner T, Graessle D. Hematopoietic cell renewal system: mechanisms of coping and failing after chronic exposure to ionizing radiation. In Radiation and Environmental Biophysics. 2008, 47(1): 63-69.

32. Nelson St. L. In Statistical Formulas in Excel. • CPA PLLC.

33. Gerstman BB. Stat Primer (Version 7.0), In Correlation. 2003, 14: 1-9.