Liver transplantation : chimerism, complications and matrix metalloproteinases

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Chapter 3

Liver chimerism after allogeneic blood stem cell transplantation

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Abstract

Background

Blood stem cells can mature into elements of many different lineages. We investigated the presence and nature of donor-derived (chimeric) cells within the liver after allogeneic stem cell transplantation.

Methods

Liver biopsy autopsy specimens were examined from nine female patients who had undergone allogeneic bone marrow (n = 6) or peripheral stem cell (n = 3) transplantation from a male donor. To identify the male origin of cells within the liver, in-situ hybridization for Y-chromosomes was performed in conjunction with CD45 staining to identify leucocytes.

Results

Hematopoietic stem cell engraftment was confirmed in all nine recipients. Histologic examination of the liver tissue sections revealed 5.6-fold more Y-chromosome- positive than CD45-positive staining cells ($P < 0.02$), indicative of considerable nonleucocytic chimerism. This was particularly observed in patients who had developed graft-versus-host disease.

Conclusions

Donor-derived cells can be found in liver tissue specimens after allogeneic stem cell transplantation. A considerable fraction of chimeric (donor-derived) cells appeared to be of nonlymphohematopoietic origin. This finding supports the theory of blood stem cells developing into liver cells of mesenchymal origin.

Introduction

Chimerism is defined in transplantation medicine as the coexistence of cells of donor and recipient origin. We previously described chimerism after transplantation of the liver and found evidence that cells of recipient origin can replace biliary epithelial cells, endothelial cells, and hepatocytes within the human liver allograft.[1] This finding can be understood only by the existence of circulating hepatic progenitor cells. To study this phenomenon further we sought to evaluate liver chimerism after allogeneic blood stem cell transplantation.

In animal studies, abundant evidence exists that bone marrow gives rise to hematopoietic as well as mesenchymal stem cells. These elements can differentiate into various cell types within the liver, such as endothelial cells, hepatocytes and bile duct epithelial cells.[2–7] In humans, the existence of circulating hepatic progenitor cells is less well established, although various studies seem to support the concept.[8–10] Two recent studies found hepatocytic differentiation of recipient-derived cells within the transplanted liver to be a rare event.[11,12] Chimerism can be demonstrated after sex-mismatched organ transplantation with the use of in-situ hybridisation for sex chromosomes. When using a Y-chromosome specific probe, cells of donor and recipient origin can be readily identified to quantitatively estimate the number of donor-derived cells. In combination with other staining techniques cells can be further differentiated.

To assess hepatic chimerism in the present study we investigated liver tissue specimens from female recipients of allogeneic stem cell transplantation (male bone marrow or peripheral blood) for the presence of donor-derived cells.

Materials and methods

Patients

From January 1994 until March 2003, we performed 100 allogeneic bone marrow transplants and 162 allogeneic peripheral blood stem cell transplantations. Female patients were selected if they received a sex-mismatched (male) transplant and had liver tissue available after transplantation. Finally, only nine female patients were included in this retrospective study based upon adequate available liver tissue specimens.

All nine had been diagnosed with a hematologic malignancy. The treatment consisted of high-dose chemotherapy followed by allogeneic bone marrow transplantation (n = 6) or allogeneic peripheral blood stem cell transplantation (n = 3). All legal and ethical criteria set out by the ethical committee were met.

Donors

All allogeneic grafts were obtained from male, HLA-matched sibling donors. To obtain peripheral blood stem cells, the donors were pretreated with recombinant human granulocyte colony stimulating factor (G-CSF, dose 10 mg/kg per day SC for 4 or 5 days) and harvesting performed by apheresis on days 5 and 6 of G-CSF administration. A minimum of 4 x 10^6 CD34 cells/kg of recipient body weight was targeted for stem cell transplantation. The stem cell graft was T-cell depleted by adding Campath to the graft. Bone marrow was obtained from donors by standard methods and was harvested under general anesthesia.

Transplantation and Follow up

Patients were conditioned with a myeloablative regimen consisting mostly of cyclophosphamide at 60 mg/kg per day IV for 2 consecutive days followed by a single dose of total body irradiation at day 1. No posttransplant graft-versus-host-disease (GVHD) prophylaxis or hematopoietic growth factors were administered. Following incubation with Campath, the stem cell product was infused intravenously on day 0.[13]

After transplantation, peripheral blood, bone marrow, or both was collected at fixed time points for morphological examination and cytogenetic analysis.

Collection of Liver Tissue Specimens

Liver tissue specimens were available from all nine patients consisting of either needle biopsy specimens or autopsy tissue. Needle biopsy specimens were obtained from four patients (1, 3, 5, 9) for diagnostic purposes because GVHD was suspected. By the time the study began, six patients were deceased. In five cases, autopsy had been performed and liver tissue was available. From each tissue specimen, consecutive sections were obtained. One was used for in situ hybridization for the Y-chromosome in combination with an Alcian blue staining. A neighboring section was stained for CD45 to identify leucocytes.

In-situ Hybridization of Y-chromosomes

For sex chromosome identification, we used in-situ hybridization using repetitive DNA probes specific for the Y-chromosome as previously described.[1,14] Briefly, probes were biotinylated by nick translation and dissolved in a 60% formamide hybridization mix- ture. Paraffin sections (6 mm thick) were cut and mounted on poly-L-lysine coated slides. Predigestion steps consisted of incubation in 1 mol/L sodium thiocyanate solution at 80°C followed by 60 to 90 minutes of treatment with 0.5% pepsin 0.1 mol/L hydrochloric acid. Hybridization was done overnight at 42°C. The hybridization reaction was visualized with avidin, biotinylated goat-anti-avidin, and avidin-peroxidase developed with diaminobenzidine. Positive and negative controls for in-situ hybridization were tissue specimens from normal male and female livers, respectively.

Additional Staining

For consecutive staining an antibody against all known isotypes of the CD45 leucocyte common antigen family present on lymphocytes, monocytes, granulocytes, and other inflammatory cells (CD45-LCA; Dako, USA) was used, as described previously.[1]

Quantification of Y-chromosome Positive and CD45 Positive Cells

All counts were performed at a magnification of 400 and expressed as the mean number of positive dots or cells/high power field (HPF). Y-chromosome-positive cells as well as CD45-positive cells were counted in a median of 13 (range 8 –16) nonoverlapping fields per biopsy.

Fluorescent In-situ Hybridization Analysis on Bone Marrow and Peripheral Blood Samples

To determine hematopoietic stem cell engraftment, peripheral blood, bone marrow samples, or both were collected at fixed time points after transplantation. Fluorescent in situ hybridization analysis (FISH) was performed and the percentage of male, donor- derived nuclei was assessed on these samples.

Statistical Analyses

A paired nonparametric test was performed (Wilcoxon's signed ranks test) to compare the results of the Y-chromosome counts with the CD45 counts. This test was applied because a normal distribution of data was not expected. The differences in cell counts in relation to the presence of GVHD were assessed using the Kruskal-Wallis test.
$P < 0.05$ was considered significant.

Results

Characteristics of Transplant Recipients

All studied recipients were females receiving stem cells from a male HLA-matched donor. Clinical characteristics concerning underlying malignant disease are outlined in Table 1. Hematopoietic stem cell engraftment was con- firmed in all nine patients.

Donor-derived Cells in Liver Tissue Specimens

Liver tissue was available from all studied patients. Table 2 shows the characteristics of the liver tissue specimens, with the histological as well as the clinical diagnosis. In three cases (3, 5, 9) histological evidence of GVHD was observed, in one other case (1) a clinical diagnosis of GVHD was made even though the liver tissue histology was unremarkable. Autopsy showed recurrent hematologic malignancy in two cases and systemic infection in three cases. The number of Y-chromosome positive cells/HPF is shown in Table 2, as well as the number of CD45 positive cells/HPF, the latter indicating the presence of infiltrating leukocytes (of donor origin).

No relation was observed between the FISH engraftment scores (Table 1) and the results from liver histology or with clinical parameters such as GVHD.

All studied liver tissue specimens showed Y-chromosome-positive cells, indicating male (donor) origin. Many of these were likely to be of hematopoietic origin, representing infiltrating leukocytes. This is supported by the finding of CD45-positive staining cells in adjacent slides (Fig 1 A and B). The absence or presence of male offspring (Table 2) was not related to the number of Y-chromosome positive cells within the liver tissue. The extent of chimerism was not related to the time elapsed between transplantation and time of biopsy.

In some cases, donor-derived cells clearly appeared to be of nonlymphohematopoietic origin, supporting the presence of true tissue chimerism (Fig 1 C-F). Paired analysis of the median Y-chromosome and CD45 counts in the successive liver tissue sections revealed a statistically significant higher number of Y+ cells (median 19.5 vs 3.5, $P < 0.02$). Interestingly, the number of Y+ cells was particularly high in the patients who developed GVHD compared to those who did not (median 29.75 vs 5, $P < 0.04$), whereas the number of CD45 cells was found to not be increased (3 vs 4, NS). Accordingly, the Y/CD45 ratio was found to be significantly higher among patients who developed GVHD (7.9 vs 1.1, $P < 0.02$).

Table 1. Clinical characteristics of the female allogeneic stem cell transplant recipients

Recipients Number	Age at Transplantation *(Years)*	Diagnosis	Type of Transplant	Time to Histology *(Month)*	Time Elapsed Since Last Transplant* *(Months)*	FISH YInterval (Bone Marrow or Blood in %)	Interval FISH Y Bone Marrow-Liver *(Weeks)*
1	46	Acute myelogeneous leukemia (AML)	Allo-BMT	14	14	98	10
2	49	Multiple myeloma (MM)	Allo-BMT	12	12	88	5
3	29	Acute myelogenous leukemia	Allo-PSCT	12	3	100	3
4	45	Acute myelogenous leukemia	Allo-BMT	4	3	99	9
5	31	Chronic myelogenous leukemia (CML)	Allo-BMT	3	3	99	1
6	39	Acute myelogenous leukemia	Allo-PCST	16	16	23	6
7	43	Acute myelogenous leukemia	Allo-BMT	10	10	NA	NA
8	17	Chronic myelogenous leukemia	Allo-BMT	3	1	99	1
9	58	Non-Hodgkin's Lymphoma (NHL)	Allo-PCST	8	1	89	0

BMT: bone marrow transplant. PSCT: peripheral blood stem cell transplantation. NA = not available.
*Last transplant: either peripheral blood stem cell transplantation (PSCT)/bone marrow transplant (BMT) or donor lymphocyte infusion/retransplant.

Table 2. Characteristics of the Liver Tissue Specimens of the Female Allogeneic Stem Cell Recipients

No	Histological Diagnosis of Liver Specimens	Out-come*	Remarks/Cause of Death (Clinical or at Autopsy)	Male Offspring	Y+ Cells Median (Range) per HPF	CD-45+ Cells Median (Range) per HPF	Y/CD-45 Ratio
1	Normal	ANED	Clinical diagnosis of GVHD Complete remission of AML	+	19.5(11-46)	2.5 (0-7)	7.8
2	Normal	DWED	Recurrent multiple myeloma	+	23 (13-38)	13(6-35)	1.8
3	Consistent with acute GVHD	DNED	Histological confirmation of chronic GVHD, DLI 3 months prior to liver histology		35.5 (14-94)	3 (0 -12)	11.8
4	Congestion and cholestasis	DNED	Systemic candidiasis, myocardial infarction	+	6 (1-12)	4 (1-10)	1.5
5	Mild inflammation	ANED	Clinical confirmation of GVHD Complete remission of CML		24 (12-36)	3 (1-6)	8.0
6	Disseminated aspergillosis	DNED	Pneumonia, disseminated aspergillosis	+	3 (1-9)	3 (0 -8)	1.0
7	Normal	DWED	Intracranial bleed, recurrent AML	+	5 (0 -11)	5 (3-9)	1.0
8	Congestion, no recurrent disease	DNED	Merantic endocarditis, invasive aspergillosis re-PCST 1 month prior to liver histology		4 (0 -11)	3.5 (0-9)	1.1
9	Acute GVHD	DWED	GVHD by histology, complete remission of NHL DLI 1 month prior to liver histology	+	58 (37-90)	21 (15-65)	2.8

*ANED = alive, no evidence of recurrent disease, DNED = deceased, no evidence of recurrent disease, DWED = deceased with evidence of recurrent disease. Abbreviations; GVHD: graft versus host disease, DLI: donor lymphocyte infusion, AML: acute myelogeneous leukemia, CML: chronic myelogenous leukemia, PSCT: peripheral blood stem cell transplantation, NHL: Non-Hodgkin's lymphoma.

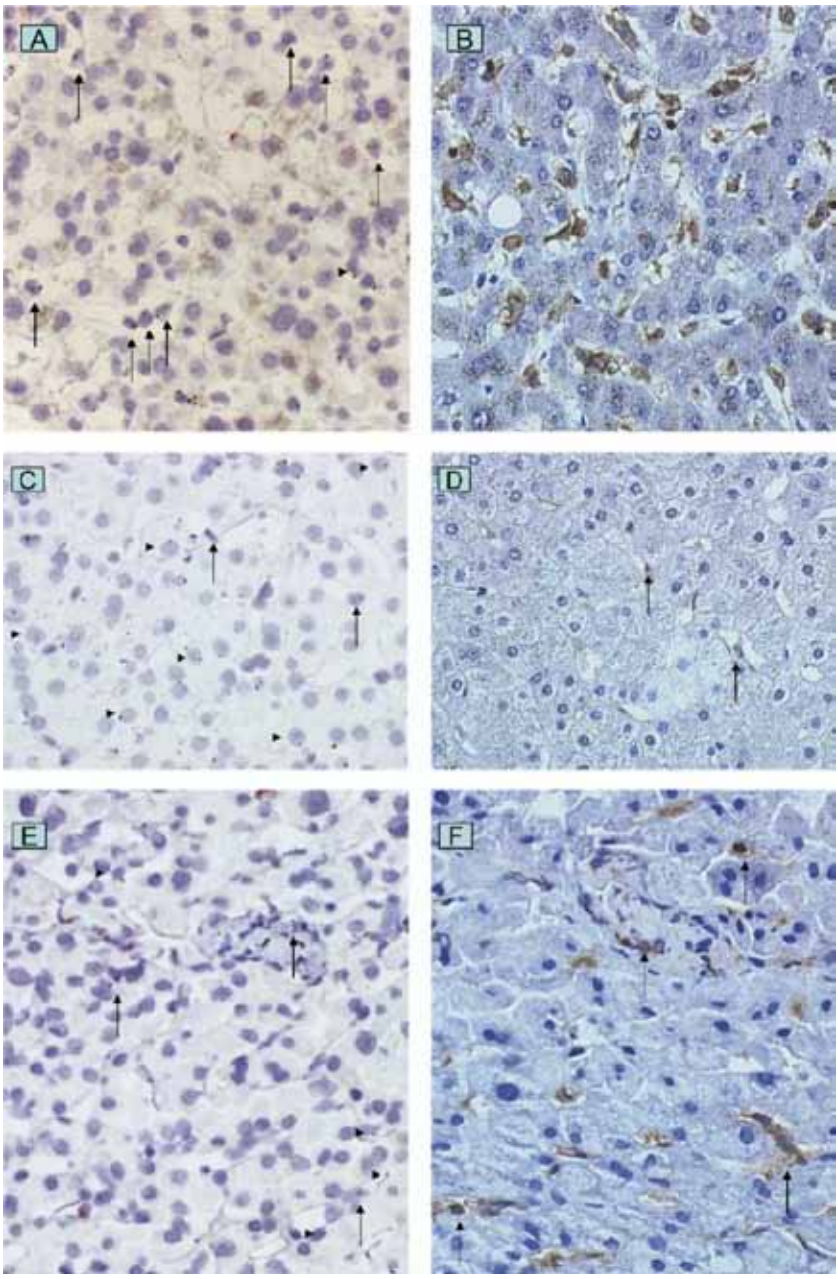

Fig 1.

In-situ hybridization for Y-chromosome (A, C, E) and parallel immunostaining for CD-45 common leucocyte antigen (B, D, F) of liver tissue sections from female patients receiving male bone marrow or blood stem cells. In several patients (example patient 2), most Y-positive cells (A, arrows) were also CD-45 positive (B, brown staining). In other patients (examples patients 1 and 5, respectively) many other cells (arrow heads), eg, hepatocytes (C and E) endothelial cells (E), were Y-positive and CD-45 negative (D and F).

Discussion

We identified liver chimerism by comparing the number of Y-chromosome-positive cells with the number of CD45-positive cells in consecutive thin tissue sections. Since the number of Y-chromosome-positive cells was significantly higher than the number of CD45-staining cells, the presence of Y-chromosomes cannot solely be attributed to infiltrating leucocytes. Furthermore, (immuno)histology confirmed Y-positive hepatocytes and endothelial cells within the liver biopsies. It strongly supports the presence of nonlymphohematopoietic cells of donor origin within the liver emerged from mesenchymal stem cells, similar to what we previously described after transplantation of the liver.[1] This indicates true chimerism within the liver. It has previously been postulated that male cells in females can result from male offspring.[15,16] In our study, however, the absence or presence of male offspring was not related to the number of Y-chromosome-positive cells.

Studies addressing chimerism in transplantation medicine mostly rely on a combination of standard histologic staining techniques and in-situ hybridization for sex chromosomes. These techniques cannot usually be applied sequentially on the same tissue section because this exposes the tissue to rough conditions, leading to loss of quality. This is especially the case in autopsy material, as in our study. Therefore, consecutive thin tissue sections are often used, as in this study. Consecutive slides are at best comparable, but never identical and therefore results need to be interpreted with some reservation.

The existence of a hepatic progenitor cell, capable of developing into hepatocytes and bile duct epithelial cells, has been investigated by many workers.[2, 9, 17-20] It has been the general belief that these hepatic stem cells (or 'oval cells') are located in the canals of Hering within the liver at the ductal plate. Fully differentiated hepatocytes themselves also possess great growth potential, and it is not known if stem cells are even required for hepatocyte regeneration. A recently postulated theory, derived from a mouse model, suggests that hepatocytes derived from bone marrow arise from cell fusion rather than by differentiation of hematopoietic stem cells.[21-24] In a study of sex-mismatched liver transplant recipients, Ng et al[12] report recipient cells constituted up to 50% of all cells in the liver allograft. Most cells showed macrophage/Kupffer cell differentiation, and only 1.6% showed hepatocytic differentiation. Again, no distinction could be made between

transdifferentiation and cell fusion. Körbling et al[8] studied recipients of peripheral blood stem cells and found donor-derived hepatocytes (up to 7%) in liver tissue specimens.

In our retrospective study, where needle biopsies were taken with a clinical suspicion of GVHD, it is remarkable that a high number of Y-chromosome positive cells was observed. One would expect this to result from a high number of infiltrating leucocytes, but this could not be confirmed because CD45 counts were not increased correspondingly. One could hypothesize that GVHD leads to hepatic tissue damage, inducing repair mechanisms,[25,26] leading to the influx of hepatic progenitor cells from the circulation and thereby to chimerism. A similar response to cellular injury may be observed in solid organ transplantation, where different types of injury, such as ischemia/reperfusion injury and rejection in liver transplantation, can initiate an immunological cascade leading to chimerism.

In conclusion, donor-derived cells may be observed in liver tissue specimens after allogeneic stem cell transplantation. A significant fraction of chimeric (donor-derived) cells appeared to be of nonlymphohematopoietic origin. This finding supports the theory of blood stem cells developing into liver cells of mesenchymal origin.

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