Bone Marrow Transplantation (2006) 37, 199–206
© 2006 Nature Publishing Group All rights reserved 0268-3369/06 $30.00
www.nature.com/bmt

ORIGINAL ARTICLE

Evaluation of the effect of transplant-related factors and tissue injury on donor-derived hepatocyte and gastrointestinal epithelial cell repopulation following hematopoietic cell transplantation

R Idilman[1], I Kuzu[2,5], E Erden[2], M Arat[3], E Soydan[3], I Soykan[1], G Akyol[4], S Karayalcin[1], H Akan[3] and M Beksac[3,5]

[1]*Department of Gastroenterology, Ankara University School of Medicine, Ankara, Turkey;* [2]*Department of Pathology, Ankara University School of Medicine, Ankara, Turkey;* [3]*Department of Hematology, Ankara University School of Medicine, Ankara, Turkey and* [4]*Department of Pathology, Gazi University School of Medicine, Ankara, Turkey*

The aim of this study was to detect donor-derived hepatocytes and gastrointestinal epithelial cells in recipients of sex-mismatched allogeneic hematopoietic cell transplants, and to assess the effect of tissue injury on the extent of the repopulation. A total of 29 paraffin-embedded biopsy samples were reviewed. Double labeling by immunohistochemistry and fluorescence *in situ* hybridization was performed. Eighty-nine percent of sex-mismatched samples with histologic evidence of injury demonstrated the presence of donor-derived hepatocytes and gastrointestinal epithelial cells (mean 2.4%). None of the hepatocytes and gastrointestinal epithelial cells in samples obtained from female recipients with female donors showed a Y chromosome signal. The proportion of donor-derived hepatocyte and gastrointestinal epithelial cells in samples with severe graft-versus-host disease was greater than that of samples with mild/moderate graft-versus-host disease ($P = 0.09$). No relationship between the source of stem cells and the population rate was detected ($P > 0.05$). We conclude that some recipient hepatocytes and gastrointestinal tract epithelial cells are replaced by donor-derived cells during tissue injury. The severity of tissue injury seems to influence on the extent of this repopulation.

Bone Marrow Transplantation (2006) **37,** 199–206. doi:10.1038/sj.bmt.1705214; published online 21 November 2005

Keywords: hematopoietic cell transplantation; repopulation; graft-versus-host disease

Introduction

Every tissue possesses stem cells that enable self renewal.[1–3] To sustain homeostasis, stem cells regulate the rate of cell production by increasing the function of cells undergoing cellular proliferation and maturation in response to tissue injury.[1–6] Recent reports suggest that hematopoietic stem cells have a capacity to transdifferentiate.[1,2,6–10]

Allogeneic hematopoietic stem cell transplantation (alloHCT) has become standard treatment for several diseases.[11] Current estimates of annual numbers of alloHCT are 12 000–15 000 worldwide, and transplant-related mortality in the first year after alloHCT averages 30%.[12] Graft-versus-host disease (GVHD), either acute (aGVHD) or chronic (cGVHD), is a major complication and contributes to transplant-related morbidity and mortality.[12–14] aGVHD occurs in approximately 40–60% of HLA-identical sibling transplants and cGVHD develops in around 50–60% of transplants.[12–14] Both acute and chronic GVHD affect the skin, liver and gut.[12–14] Since histological findings of GVHD are nonspecific, diagnosis often requires both clinical and histological criteria.[12–14] The induction of increased histocompatibility antigens and leukocyte adhesion molecule expression on tissues such as bile ducts, gastrointestinal mucosa and epidermis identifies these cells as targets for alloreactive donor T cells. Such T cells proliferate and secrete inflammatory cytokines (IL-2, IL-1 and TNF-α which induce tissue injury.[13,15,16]

Bone marrow (BM)-derived stem cells have been shown to engraft and populate hematopoietic as well as non-hematopoietic tissues during injury and repair. However, the contribution of BM cells to the generation of new hepatocytes and gastrointestinal epithelial cells and the role of the severity of the tissue injury on such responses are controversial. The aims of this study were to investigate the presence of donor-derived hepatocytes and gastrointestinal epithelial cells in sex-mismatched individuals following alloHCT, and to evaluate the effect of tissue injury, occurring as a result of the conditioning regimen or GVHD on the extent of the repopulation.

Correspondence: Dr R Idilman, Department of Gastroenterology, Ankara University School of Medicine, Ibn'i Sina Hospital, Sihhiye, Ankara 06100, Turkey.
E-mail: idilman@dialup.ankara.edu.tr
[5]These authors are members of the Turkish Academy of Sciences (TUBA)
Received 13 May 2005; revised 29 June 2005; accepted 12 August 2005; published online 21 November 2005

Materials and methods

A total of 29 paraffin-embedded liver (*n*:17) and gastrointestinal (*n*:12) biopsy samples of 28 recipients (male/female ratio: 16/12; median age: 31.0 years, range: 14–53 years) were investigated. All 28 individuals received alloHCT from their HLA-identical siblings at the Ankara University School of Medicine, Stem Cell Transplantation Unit. The median donor age was 30.0 years (range: 15–56 years). Sixteen of the 28 recipients had an ABO-mismatched donor. The source of the stem cells was peripheral blood (PB) in 21 and BM in seven recipients. Twenty-two recipients were conditioned with an ablative regimen consisting of busulfan (16 mg/kg) and cyclophosphamide (120 mg/kg). The remaining six received fludarabine-based reduced-intensity chemotherapy. All received short-term methotrexate and cyclosporine (Cs-A) (myeloablative) or mycophenolate mofetil and Cs-A (reduced intensity) to prevent GVHD after alloHCT. The hematologic and demographic characteristics of the recipients and donors are shown in Table 1a and b.

Biopsy indications

A liver biopsy was performed when liver injury tests and cholestatic liver enzymes were found to be increased. Gastrointestinal mucosal biopsies were performed to identify the etiological cause of nausea, vomiting, abdominal discomfort, inability to eat and diarrhea. No sequential liver and gastrointestinal tract biopsies were performed. Paraffin sections were stained by hematoxylin–eosin (HE). The diastase-digested periodic acid-Schiff and Masson's trichrome stains were utilized to enable a more extensive histopathological evaluation of the changes in the liver especially in terms of the bile duct damage and tissue fibrosis. Only HE-stained paraffin sections of the gastrointestinal tract biopsies were examined.

Table 1 Patient characteristics

	Age	*Gender of recipients*	*Gender of donors*	*Primary disease*	*Transplant type*	*Conditioning regimen*	*Time from Tx to biopsy (months)*	*CD 34 count × 10^6/kg*	*Donor type chimerism*[a] *(%)*	*Hepatocyte repopulation (%)*
(a) Characteristics of AlloHCT recipients with respect to hepatocyte repopulation										
Case 1[b]	39	XX	XX	AML	PB	AB	20	12.4	NA	0
Case 2	33	XX	XY	CML	PB	AB	6	2.8	NA	0
Case 3	23	XY	XX	ALL	PB	AB	5	6.1	100	0.8
Case 4	36	XX	XY	CML	PB	AB	10	8.1	NA	0.8
Case 5	43	XY	XX	CML	BM	AB	19	0.4	100	0.8
Case 6a	43	XX	XY	CML	PB	RI	4	5.4	NA	1.2
Case 7	48	XY	XX	AML	PB	AB	11	6.4	100	1.5
Case 8	39	XX	XY	CML	PB	RI	4	3.9	92	1.6
Case 9	26	XY	XX	AML	PB	AB	9	4.5	100	1.6
Case 10	31	XY	XX	CML	BM	AB	3	2.0	100	2.0
Characteristics of AlloHCT recipients with respect to liver biopsy time										
Case 11	24	XY	XX	AML	PB	AB	5	5.6	97	2.4
Case 12	31	XY	XX	AML	PB	AB	9	7.5	100	2.4
Case 13	25	XY	XX	CML	PB	AB	6	3.0	NA	2.7
Case 14	34	XY	XX	CML	PB	AB	11	7.4	NA	3.9
Case 15	39	XY	XX	AML	PB	AB	7	5.4	97	4.0
Case 16a	24	XY	XX	AML	PB	AB	6	4.0	100	4.2
Case 17	53	XY	XX	MDS	PB	RI	5	2.9	100	5.6

	Age	*Gender of recipients*	*Gender of donors*	*Primary disease*	*Tx type*	*Conditioning regimen*	*Time from Tx to biopsy*	*CD 34 count × 10^6/kg*	*Donor type chimerism*[a] *(%)*	*Epithelial repopulation (%)*
(b) Characteristics of AlloHCT recipients with respect to gastrointestinal tract biopsy time										
Case 18	14	XX	XY	AML	PB	AB	1	13.6	NA	0
Case 19[b]	33	XX	XX	CML	BM	AB	3	1.1	NA	0
Case 20	28	XX	XY	CML	BM	AB	1	1.8	94	0.8
Case 21	19	XX	XY	AML	PB	AB	1	4.8	NA	0.8
Case 22	22	XY	XX	CML	BM	AB	1	0.8	92	1.2
Case 23	46	XX	XY	MM	BM	AB	2	3.3	NA	1.2
Case 24	31	XY	XX	CML	PB	AB	1	5.6	100	2.0
Case 25	17	XX	XY	ALL	PB	AB	7	1.9	100	2.0
Case 26	28	XY	XX	NHL	PB	RI	2	6.0	100	3.2
Case 27	30	XY	XX	CML	PB	RI	6	11.4	100	3.2
Case 28	20	XX	XY	HD	PB	RI	5	4.8	100	3.4
Case 6b	43	XX	XY	CML	PB	RI	1	5.4	77	6.4

NA = not available.

[a]For donor type chimerism, after the isolation of genomic DNA from peripheral blood, 11 STR loci were amplified by Amp FISTRA SGM plus PCR Amplification Kit (Perkin Elmer Bio-systems, USA). The amplified products were analyzed by Gene Scan Analyzer following capillary electrophoresis.

[b]Control; PB = peripheral blood; BM = bone marrow; AB = ablative; RI = reduced intensity; ALL = acute lymphoblastic leukemia; AML = acute myelogenous leukemia; CML = chronic myelogenous leukemia; MDS = myelodysplastic syndrome.

Diagnosis of the liver tissue injury

Conditioning regimen-related drug hepatotoxicity. Conditioning regimen-related hepatotoxicity was defined as an elevation of aminotransferases levels (greater than 1.3 times the upper limit of normal) occurring with an increase in the serum bilirubin level above the upper limit of normal, with or without an elevation of the level of cholestatic enzymes occurring from day −7 to +30 post-alloHCT screening.[17] Serum alanine aminotransferase (ALT, normal: 10–37 IU/l), aspartate aminotransferase (AST, normal: 10–37 IU/l), gamma glutamyl transpeptidase, alkaline phosphatase and bilirubin levels were determined at days −7, −3, 0 and at days 1, 3, 7, 14, 21 and 30 post transplantation.

GVHD occurring in the liver. Hepatic GVHD was defined as an elevation of serum alkaline phosphatase levels (greater than two times the upper limit of normal) occurring in association with an increase in the serum bilirubin level above the upper limit of normal, with or without an increase in the serum aminotransferases levels at day +100 post-alloHCT. In each case, the diagnosis of hepatic GVHD occurring in the liver was confirmed by liver biopsy. The histologic criteria utilized were the finding of bile duct damage and/or ductopenia with infiltration of the smaller bile ducts with lymphocytes, portal area expansion with both lymphocytes and plasma cells and cholestasis as previously published.[18]

The limited/extensive GVHD classification was used for the clinical and histological grading of cGVHD of the liver. For histopathological grading, a modified scheme introduced by Shulman *et al*.[19] was applied. The modified-histopathological GVHD grading scheme is shown in Table 2. Based on the total score, liver biopsy samples were divided into two distinct groups. Samples with histopathological GVHD grading of less than 6 were considered as mild/moderate GVHD of the liver. Scores between 6 and 10 were considered as severe GVHD.

GVHD of the gastrointestinal tract. Gastrointestinal tract biopsy was performed in endoscopically abnormal areas of the gastrointestinal tract. Gastrointestinal biopsies were stained with HE, and graded for the presence and severity of GVHD using the criteria of McDonald *et al*.[20] For statistical analysis, grades were sorted into two groups according to the severity of the epithelial damage. Grades I–II, consisting of prominent inflammatory reaction and mild epithelial damage, were grouped as 'mild GVHD'. Grade III and IV changes with severe epithelial damage, consisting of drop out of crypts, and denudation of epithelial cells were grouped as 'severe GVHD'.

Immunohistochemical staining. Sections (4 μm) of formalin-fixed paraffin-embedded tissues were immunostained using monoclonal mouse anti-human hepatocyte antibody (Clone OCH1E5, DAKO, Glostrup, Denmark) at a 1:30 dilution to identify hepatocyte and anti-human cytokeratin 7 (Clone K72.7, DBS CA, USA,) and at a 1/100 dilution to identify bile duct epithelial cells. Anti-human cytokeratin (Novacastra, UK, clone: 5D3 + LP34) at a 1:50 dilution was used to demonstrate gastrointestinal epithelial cells, and a mixture of anti-CD45 at a 1:20 dilution (Novacastra, UK, clone: RP2/18 + RP2/22) and anti-CD15 at a 1:50 dilution (Neomarkers, USA, clone 15CO2) was used to identify lymphocytes and leukocytes. These primary antibodies were incubated for 60 min at room temperature on two separate sections for which HRP-conjugated goat anti-mouse secondary antibody followed by DAB substrate for light microscopic visualization of the cells was used.

In situ *hybridization.* The immunostained sections were prepared for fluorescence *in situ* hybridization (FISH) by sequential acid incubation, denaturation, and proteinase K digestion steps. After formamide denaturation and formalin fixation, the slides were dehydrated in graded ethyl alcohols and air dried. FISH was performed on

Table 2 Modified histopathological grading of GVHD of the liver

	Histopathological features	*Scores*
Hepatic architecture	Normal	0
	Central, portal and periportal fibrosis	1
	Bridging fibrosis, cirrhosis	2
Hepatocytes and lobular features	Normal	0
	Endothelialitis, lobular disarray, ballooning, acidophilic bodies in lobules, fatty changes, enlarged pigmented hepatocytes, cholestasis, bile in centrilobular hepatocytes	1
	Confluent centrilobular collapse, bile plugs	2
Portal triad	Normal	0
	Edema, lymphocyte infiltration with/without portal fibrosis	1
	Spillover of limiting plate, piecemeal necrosis with/without periportal fibrosis	2
	Portal–portal bridging, collapse with/without bridging fibrosis	3
Bile ducts	Normal	0
	Nuclear pleomorphism, nuclear enlargement, cytoplasmic vacuolization-swelling, Cytoplasmic eosinophilia, increased nucleus/cytoplasm ratio	1
	Bile duct distribution	2
	Cholangiole proliferation, bile duct loss	3

Samples with histopathological GVHD grading of less than 6 were considered as mild/moderate GVHD of the liver. Scores between 6 and 10 were considered as having severe GVHD.

pretreated slides using spectrum orange-labeled DXZ1 (Xp11, X-q11.1) (CEPX) Alpha Satellite and spectrum green-labeled DYZ1 (Y) (Yq1 2) probes (Vysis Inc., Downers Grove, IL-USA) for labeling X and Y chromosomes. After the application of probes, covered and sealed slides were denatured 10 min at 90°C and hybridized 12–18 h at 42°C using a hybrid incubation chamber (Vysis Inc., Downers Grove, IL, USA). DAPI was performed for nuclear counter-staining (Vysis Inc., Downers Grove, IL, USA).

Tissue analysis. The slides were viewed and analyzed systematically using an image analyzer system (Olympus microscope with 3X61 motorized, Cyto-Vision, Applied Imaging). Every field was z-scanned at seven levels in order to eliminate misinterpretation due to any super- or juxtaposition of the cells on dark field for X and Y chromosomal signals. Only the cells with two nuclear spots were considered for interpretation. At the same time, immunophenotypes of either hepatocytes or ductal cells for liver biopsies, and mucosal epithelial cells and inflammatory cells on GI biopsy sections were assessed on bright field for confirmation of the cell type. The percentage of donor type chimerism was calculated by counting 250 cells on each section. All available ductal cells in each liver biopsy were counted.

Statistical analysis. The data were analyzed using the Student's *t*-test and χ^2 test. A *P*-value of less than 0.05 was considered significant.

Results

Among 29 biopsy samples, 27 were sex-mismatched biopsy samples and two were sex-matched biopsy samples (female to female), which were used as control. Sixteen of the 27 sex-mismatched samples were obtained from male recipients who had female donors and 11 were obtained from female recipients who had male donors. Among 27 sex-mismatched biopsy samples, 16 were liver and 11 were gastrointestinal tract (three antrum of stomach, six duodenum and two rectum). One recipient with a sex-mismatched donor had both a liver and a gastrointestinal biopsy. Ten of the 12 female recipients had no prior history of male pregnancy. None of the biopsies had had normal tissue. However, the extent of tissue injury was variable.

Donor-derived hepatocyte and gastrointestinal cell in AlloHCT recipients

The mean time from transplantation to biopsy was 5.9 months (range: 1–20 months). Twenty-four of the 27 sex-mismatched samples (88.9%) demonstrated donor-derived hepatocyte and gastrointestinal epithelial cell repopulation with a mean of 2.4% (range: 0.8–6.4%).

Hepatocyte. The mean time from transplantation to biopsy was 8.2 months (range: 3–20 months). Fifteen of these 16 sex-mismatched samples (93.8%) demonstrated donor-derived hepatocyte repopulation (DDHR) with a mean of 2.4% (range: 0.8–5.6%) of the hepatocytes being of donor origin (Figure 1a and b). DDHR was not demonstrated in the remaining one sex-mismatched sample; however, donor-derived unspecified sinusoidal cells were seen in the liver sinusoids of this individual. None of the hepatocytes of sex-matched liver biopsy samples obtained from female recipients with female donors manifested a Y chromosome signal.

Donor gender did not influence the presence or proportion of DDHR. Three of the four samples obtained from female recipients with male donors demonstrated DDHR with a mean proportion of 1.2% of the hepatocytes (range: 0.8–1.6%) (Table 3), while all of the 12 samples obtained from male recipients with a female donor demonstrated DDHR with a mean proportion of 2.7% of the hepatocytes (range: 0.8–5.6%) (1.2 vs 2.7%, $P>0.05$).

Bile duct epithelial cells. Eight of the 17 sex-mismatched liver biopsy samples had available portal areas with

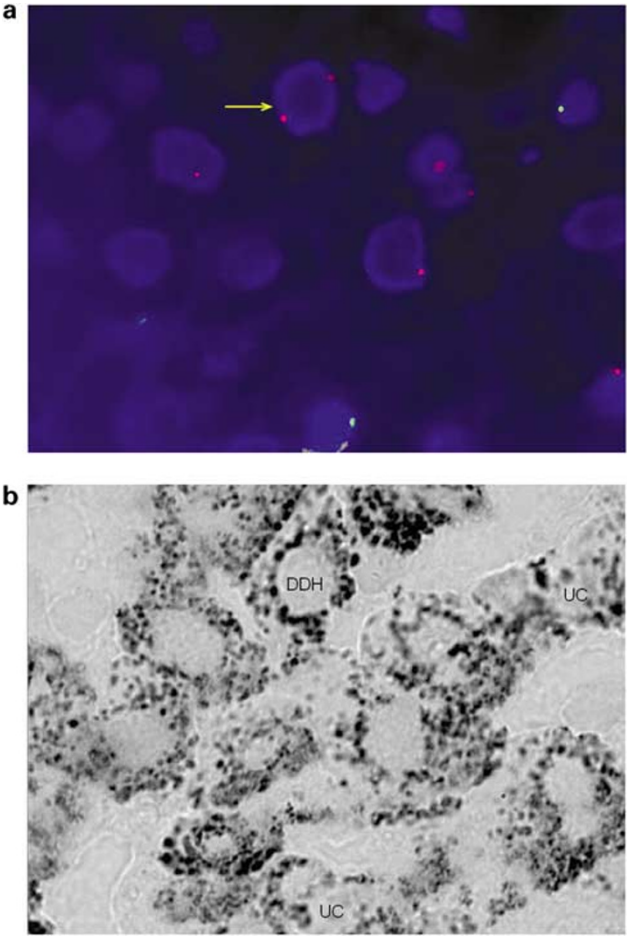


Figure 1 (**a**) Donor-derived hepatocyte repopulation in liver biopsy sample of male recipient with a female donor. Nuclear red spots were interpreted as X chromosomal signals, green spots as Y chromosomal signals. The arrow indicates the apparent repopulated hepatocytes. (**b**) Cytoplasmic positivity with hepatocyte antigen by immunohistochemistry on the same section. (DDH: donor-derived hepatocyte, UC: unspecified sinusoidal cell).

recognizable bile ducts. Two to six portal areas were available for each of these samples. A total of 136 bile duct epithelial cells (mean: 19.4 epithelial cells per sample; range: 4–36 epithelial cells/sample) were counted. None of the bile duct epithelial cells expressed a different nuclear signal from that of the recipient.

Gastrointestinal tract epithelial cell. The mean time from transplantation to biopsy was 2.5 months (range: 1–7 months). Nine of the 11 sex-mismatched gastrointestinal biopsy samples (81.8%) demonstrated donor-derived gastrointestinal tract epithelial cell repopulation (DDGECR) with a mean of 2.5% (range: 0.8–6.4%) (Figure 2a and b). The remaining two sex-mismatched samples did not demonstrate DDGECR, but donor-derived inflammatory cells were present in the biopsies. Furthermore, none of the gastrointestinal epithelial cell of sex-matched biopsy samples obtained from female recipients with female donors manifested a Y chromosome signal.

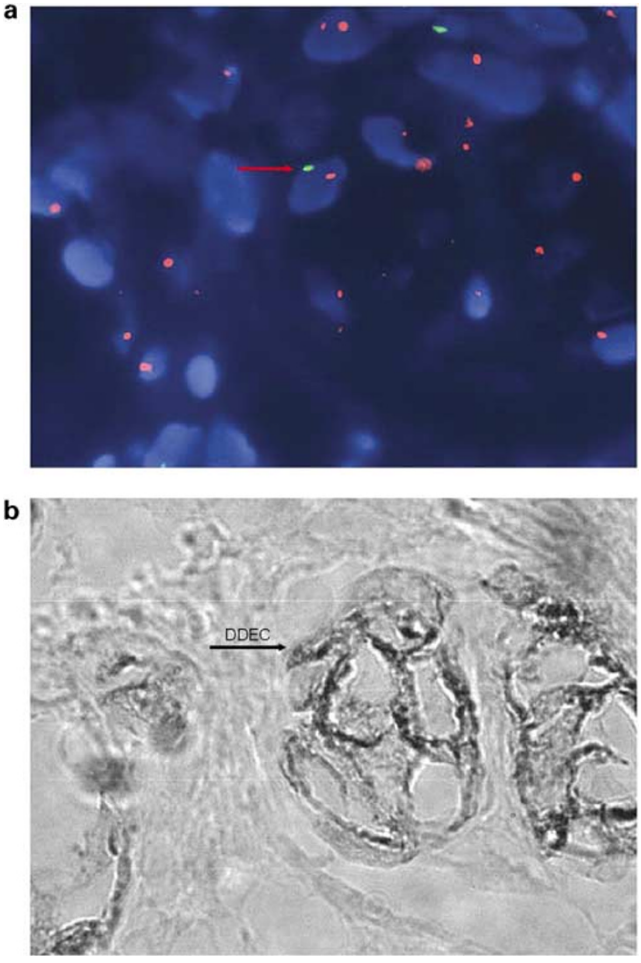


Figure 2 (**a**) Donor-derived gastrointestinal epithelial cell repopulation in a gastrointestinal tract mucosal biopsy sample of a female recipient with a male donor. Nuclear red spots were interpreted as X chromosomal signals, green spots as Y chromosomal signals. The arrow indicates the apparent repopulated epithelial cell. (**b**) Cytoplasmic positivity with anti-human cytokeratin by immunohistochemistry on the same section (DDEC: donor-derived epithelial cell).

Donor gender did not influence either the presence or proportion of DDGECR. Six of the seven sex-mismatched samples obtained from female recipients with a male donor demonstrated DDGECR with a mean proportion of 2.3% (range: 0.8–6.4%) (Table 3), whereas three of the four samples obtained from male recipients with a female donor demonstrated DDGECR with a mean proportion of 2.8% (range: 2.0–3.2%) (2.3 vs 2.8%, $P>0.05$).

Tissue injury and donor-derived cell repopulation

Eight of the 16 recipients with sex-mismatched donors had shown drug-related liver toxicity during the first month after alloHCT. Serum AST and ALT levels increased significantly (serum AST level from 30.3 ± 16.1 to 55.9 ± 35.3 IU/l at day 0 to 43.8 ± 19.4 IU/l at day $+7$; serum ALT level from 31.3 ± 13.8 to 65.6 ± 41.3 IU/l at day 0 to 129.4 ± 49.8 IU/l at day $+7$) (baseline ALT vs day $+7$ ALT, $P=0.0001$). The mean time from onset of liver function test abnormalities to liver biopsy was 2.7 months (range: 1 day–12 months). The time from liver function test abnormalities to liver biopsy did not influence either the presence or proportion of DDHR ($P>0.05$).

GVHD. Ten of the 11 sex-mismatched gastrointestinal biopsy samples were identified as having acute GVHD. Five were graded as mild GVHD and five were graded as severe GVHD. The remaining one was diagnosed as mucositis. The proportion of DDGECR in samples with severe GVHD of the gastrointestinal tract was slightly higher than that of samples with mild GVHD of the gastrointestinal tract (mean proportion 3.1% (range: 0.8–6.4%) vs 1.1% (range: 0–2.8%), $P=0.09$) (Table 3, Figure 3). All five gastrointestinal samples with severe GVHD demonstrated DDGECR, whereas three of the five gastrointestinal samples with mild GVHD demonstrated DDGECR (5/5 vs 3/5, $P>0.05$) (Table 3).

With histopathological GVHD grading of the liver, 10 of the 16 sex-mismatched liver biopsy samples were graded as mild/moderate GVHD, whereas the remaining six samples were graded as severe GVHD. DDHR in samples with severe GVHD of the liver were slightly more common than that of samples with mild/moderate GVHD of the liver (6/6, 3.0% vs 9/10, 1.7%, respectively; $P=0.09$) (Table 3, Figure 3).

With clinical GVHD grading, seven of the 16 recipients with sex-mismatched donors had limited cGVHD. The remaining nine had extensive cGVHD. There was no significant difference in terms of the presence or proportion of DDHR between liver biopsy samples with limited and extensive cGVHD (2.4% (range: 0–5.6%) vs 2.1% (range 0.8–4.2%), $P>0.05$). Six of the seven samples with limited cGVHD demonstrated DDHR, whereas all of the nine samples with extensive cGVHD demonstrated DDHR (Table 3).

The type of conditioning regimen did not influence either the presence or proportion of DDHR. The presence or proportion of DDHR showed no significant difference in individuals with hepatic GVHD with/without conditioning regimen-related drug hepatotoxicity (8/8, mean proportion, 2.1% vs 7/8 mean proportion, 2.4%; $P>0.05$) (Table 3).

Table 3 The presence and intensity of 'donor-derived hepatocyte and gastrointestinal epithelial cell repopulation' in sex-mismatched alloHCT

	Presence of repopulation n (%)	*Mean proportion of repopulation (mean, %) (range, %)*	*P*
Gender			
Male donation to female recipient	9/11 (82%)	2.0% (0.8%–6.4%)	>0.05
Female donation to male recipient	15/16 (94%)	2.7% (0.8%–5.6%)	
Stem cell source			
Bone marrow	5/6 (83%)	1.4% (0.8%–2.0%)	>0.05
Peripheral blood	19/21 (91%)	2.7% (0.8%–6.4%)	
Tissue type			
Liver	15/16 (94%)	2.4% (0.8%–5.6%)	>0.05
Gastrointestinal tract	9/11 (82%)	2.5% (0.8%–6.4%)	
Severity of GVHD of the liver			
Limited/mild-moderate	6/7 (86%)/9/10 (90%)	2.4% (0%–5.6%)/1.7%	=0.09*
Extensive/severe	9/9 (100%)/6/6 (100%)	2.1% (0.8%–4.2%)/3.0%	
Hepatic GVHD plus drug-related hepatotoxicity			
Yes	8/8 (100%)	2.1% (0.8%–4.0%)	>0.05
No	7/8 (88%)	2.4% (0%–5.6%)	
Severity of GVHD of the GI tract			
Mild	3/5 (60%)	1.1% (0%–2.8%)	=0.09
Severe	5/5 (100%)	3.1% (0.8%–6.4%)	

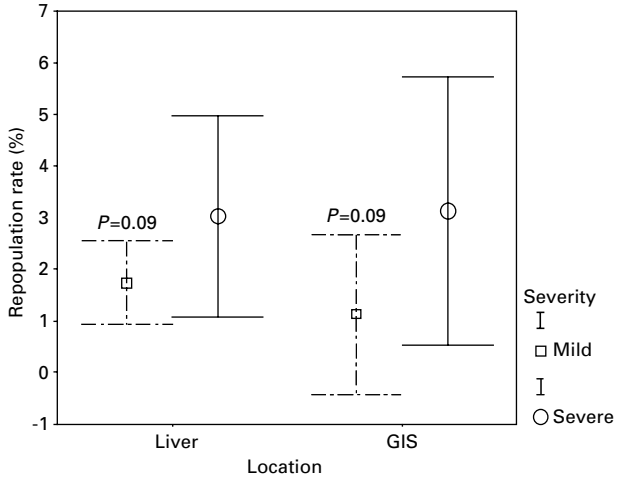


Figure 3 The effect of the severity of the tissue injury on the rate of donor-derived cell repopulation.

Stem cell source. The source of the stem cells used for transplantation had no effect on the repopulation rate ($P>0.05$). Nineteen of the 21 tissue samples obtained from PB recipients demonstrated donor-derived hepatocyte and gastrointestinal epithelial cell repopulation, while five of the six tissue samples obtained from BM recipients demonstrated donor-derived hepatocyte and gastrointestinal epithelial cell repopulation (mean proportion 2.7% (0.8–6.4%) vs 1.4% (0.8–2.0%), $P>0.05$) (Table 3).

Discussion

In the present study, 89% of the sex-mismatched tissue samples demonstrated donor-derived hepatocyte and gastrointestinal epithelial cell repopulation with a mean proportion of 2.4%. Among these samples, DDHR was observed in 94% of the liver biopsy samples with a mean proportion of 2.4%. DDGECR was observed in 82% of the gastrointestinal biopsy samples with a mean proportion of 2.5%. This is the first study including a large number of cases (sample number: 29) demonstrating donor-derived hepatocyte and gastrointestinal epithelial cell repopulation when compared to the previous reports by Theise *et al.* (sample number: 6) and Korbling *et al.* (sample number: 12).[1,7] Donor gender did not influence the presence or proportion of donor-derived hepatocyte and gastrointestinal epithelial cell repopulation (for hepatocytes, mean proportion male to female, 1.2% vs female to male, 2.7%; for gastrointestinal epithelial cell repopulation, mean proportion male to female, 2.3% vs female to male, 2.8%, respectively; $P>0.05$). These data suggest that donor-derived hepatocyte and gastrointestinal epithelial cell repopulation occurs in alloHCT independent of donor gender.

Several studies have addressed the question of the extent of donor-derived hepatocyte and gastrointestinal epithelial cell repopulation.[1–4,7,21,22] In the present study, no relationship between tissue injury due to either conditioning regimen or the extent of GVHD and the presence and proportion of donor-derived hepatocyte and gastrointestinal epithelial cell repopulation was observed, although the proportion of donor-derived hepatocyte and gastrointestinal epithelial cell repopulation in individuals with severe GVHD was slightly greater. Kanazawa *et al.*[23] and Korbling *et al.*[7] reported that the frequency of DDHR in recipients is unrelated to liver injury in both experimental models and humans. In contrast, Theise *et al.*[1] reported that the extent of engraftment in sex-mismatched graft seems to correlate with the severity of tissue injury. When tissue injury was mild, a low level of stem cell repopulation

occurred. On the other hand, with severe tissue injury, a higher level of stem cell repopulation occurred.[1] We believe that these controversial findings may be the consequence of the nature of the injury, the length of the period between injury and biopsy, or the sensitivity of the techniques investigators have used.

The origin of these 'repopulated cells' and the manner in which they generate hepatocytes and gastrointestinal tract epithelial cells are controversial. Basic concepts on stem cell biology including plasticity, transdifferentiation and fusion have all been reported.[1,2,5,22–29] Recent progresses in stem cell studies have indicated that certain mammalian cells maintain a high degree of plasticity for multilineage cell differentiation.[25,26] The plasticity is supported by the finding of intrahepatic chimerism in transplant recipients.[25,26] Theise *et al*. investigated the question of whether hepatocytes could be generated from the BM cells of the recipient.[1] Alison *et al*.[2] and Korbling *et al*.[7] addressed this question in the liver of recipients with sex-mismatched donors and we made our own observations as well.[30] In these studies, the frequency of BM-derived hepatocytes in the liver of the recipients ranged from 0.5 to 8% without using a correction factor. Two other studies of transplants were not able to detect BM-derived cells in the liver.[31,32] In the present study, in addition to being able to demonstrate donor-derived hepatocyte and gastrointestinal epithelial cell repopulation, such repopulation was found to be slightly greater in recipients of PB transplantation as compared to recipients of BMT (2.7 vs 1.4%, $P=0.08$). Based on the results of all of these studies, it appears that donor-derived hepatocyte and gastrointestinal epithelial cell repopulation occurs, but at a low frequency.

Cell fusion has been reported as an alternative mechanism responsible for cell fate changes.[27–29] *In vitro* fusion between embryonic stem cells and somatic cells has been described[27–29] but its frequency is low. Terada *et al*.[29] were not able to demonstrate fusion in their hematopoietic stem cell (HSC) culture model. They have suggested that HSCs may be incapable of fusion.[29] The frequency of *in vivo* fusion between transplanted marrow cells and injured liver in a model of tyrosinemia was higher than in other circumstances or models. These investigators have suggested that plasticity occurs first and is followed later by fusion.[28] Unfortunately, the present work does not discriminate among the various potential mechanisms of cellular repopulation.

Although donor-derived hepatocyte and gastrointestinal tract epithelial cell repopulation was demonstrated in this study, we were unable to demonstrate cholangiocyte repopulation. This finding is in contrast with the data of other studies, which report cholangiocyte engraftment in all samples but at different proportions.[1,33] Our failure to demonstrate this phenomenon may be a result of the limited number of portal areas examined in the liver biopsy samples available in the present study.

Women who have not undergone transplantation can harbor a Y chromosome in their liver if they have had a previous male child pregnancy. This has been documented by several different investigators.[34,35] In the present study, 10 of the 12 recipients had no male child or abortion of a male gestational product. This observation supports that the Y-chromosome harboring hepatocytes in the female liver grafts in the present study originated from male donor-derived stem cells.

Based on the results of this study, we conclude that some hepatocytes and gastrointestinal tract epithelial cells of recipients with sex-mismatched donors are replaced by donor-derived stem cells. Such repopulation occurs at a low frequency. The severity of tissue injury seems to have a minimal effect on the proportion of donor-derived hepatocyte and gastrointestinal epithelial cell repopulation. We suggest that the origin of these repopulated cells in the post transplant period is donor-derived stem cells. The fate of the donor-derived hepatocyte and gastrointestinal epithelial cell repopulation can only be evaluated using systematically performed tissue biopsies in longitudinal studies.

Acknowledgements

We thank Muhip Ozkan, MD, Ankara University Agriculture Faculty, Department of Biometry-Genetics, for his assistance with statistical analysis; Ajlan Tukun, MD, Ankara University School of Medicine, Department of Medical Genetics, for her generous help in using the image analyzer system; and Yasemin Sahin, Ankara University Biotechnology Institute, and Klara Dalva Ankara University School of Medicine, Hematology Laboratory, for their technical assistance. Ramazan Idilman and Mutlu Arat have been supported by the Turkish Academy of Sciences, in the framework of the Young Scientist Award Program (EA-TUBA-GEBIP/2001-1-1, 2004-1-1).

References

1 Theise ND, Nimmakayalu M, Garner R, Illei PB, Morgan G, Teperman L *et al*. Liver from bone marrow in humans. *Hepatology* 2000; **32**: 11–16.
2 Alison MR, Poulsom R, Jeffery R, Dhillon AP, Quaglia A, Jacob J *et al*. Hepatocytes from non-hepatic adult stem cells. *Nature* 2000; **406**: 257.
3 Forbes S, Vig P, Poulsom R, Thomas H, Alison M. Hepatic stem cells. *J Pathol* 2002; **197**: 510–518.
4 Theise ND, Badve S, Saxena R, Henegariu O, Sell S, Crawford JM *et al*. Derivation of hepatocytes from bone marrow cells in mice after radiation-induced myeloablation. *Hepatology* 2000; **31**: 235–240.
5 Korbling M, Estrov Z. Adult stem cells for tissue repair: a new therapeutic concept. *N Engl J Med* 2003; **349**: 570–582.
6 Brittan M, Wright NA. Stem cell in gastrointestinal structure and neoplastic development. *Gut* 2004; **53**: 899–910.
7 Korbling M, Katz RL, Khanna A, Ruifrok AC, Rondon G, Albitar M *et al*. Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. *N Engl J Med* 2002; **346**: 738–746.
8 Orlic D, Hill JM, Arai AE. Stem cells for myocardial regeneration. *Circ Res* 2002; **91**: 1092–1102.
9 Akiyama Y, Radtke C, Honmou O, Kocsis JD. Remyelination of the spinal cord following intravenous delivery of bone marrow cells. *Glia* 2002; **39**: 229–236.
10 Mahmood A, Lu D, Lu M, Choop M. Treatment of traumatic brain injury in adult rats with intravenous administration of human bone marrow stromal cells. *Neurosurgery* 2003; **53**: 697–702.
11 Thomas ED. Bone marrow transplantation: a review. *Semin Hematol* 1999; **36**: 95–103.

12 Akpek G. Graft-versus-host disease: an evidence-based management guideline. *Turk J Hematol* 2003; **20**: 123–142.
13 Ferrara JLM, Deeg HJ. Graft-versus-host disease. *N Engl J Med* 1991; **324**: 667–674.
14 Sullivan KM, Agura E, Anasetti C, Appelbaum F, Badger C, Bearman S *et al*. Chronic graft-versus-host disease and other late complications of bone marrow transplantation. *Semin Hematol* 1991; **28**: 249–258.
15 Storek J, Gooley T, Siadak M, Bensinger WI, Maloney DG, Chauncey TR *et al*. Allogeneic peripheral blood stem cell transplantation may be associated with a high risk of chronic graft-versus-host disease. *Blood* 1997; **90**: 4705–4709.
16 Atkinson K, Horowitz MM, Gale RP, van Bekkum DW, Gluckman E, Good RA *et al*. Risk factors of chronic graft-versus-host disease after HLA-identical sibling bone marrow transplantation. *Blood* 1990; **75**: 2459–2464.
17 Bearman SI, Appelbaum FR, Buckner CD, Petersen FB, Fisher LD, Clift RA *et al*. Regimen-related toxicity in patients undergoing bone marrow transplantation. *J Clin Oncol* 1988; **6**: 1562–1568.
18 Shulman HM, Sullivan KM, Weiden PL, McDonald GB, Striker GE, Sale GE *et al*. Chronic graft-versus-host syndrome in man: a long-term clinico-pathologic study of 20 Seattle patients. *Am J Med* 1980; **69**: 204–217.
19 Shulman HM, Sharma P, Amos D, Fenster LF, McDonald GB. A coded histologic study of hepatic graft-versus-host disease after human bone marrow transplantation. *Hepatology* 1988; **8**: 463–470.
20 McDonald GB, Sale GE. The human gastrointestinal tract after allogeneic marrow transplantation. In: Sale GE, Shulman HM (eds). *The Pathology of Bone Marrow Transplantation*. Masson: New York, 1984, pp 77–103.
21 Fausto N. Liver regeneration and repair: hepatocytes, progenitor cells, and stem cells. *Hepatology* 2004; **39**: 1477–1487.
22 Shackel N, Rockey DC. Stem cells and liver disease: promise laced with confusion and intrigue. *Gastroenterology* 2004; **127**: 346–348.
23 Kanazawa Y, Verma IM. Little evidence of bone-marrow-derived hepatocytes in the replacement of injured liver. *Proc Natl Acad Sci USA* 2003; **100**: 11850–11853.
24 Petersen BE, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N *et al*. Bone marrow as a potential source of hepatic oval cells. *Science* 1999; **284**: 1168–1170.
25 Jang YY, Collector MI, Baylin SB, Diehl AM, Sharkis SJ. Hematopoietic stem cells convert into liver cells within days without fusion. *Nat Cell Biol* 2004; **6**: 532–539.
26 Camargo FD, Chambers SM, Goodell MA. Stem cell plasticity: from transdifferentiation to macrophage fusion. *Cell Prolif* 2004; **37**: 55–65.
27 Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM, Fike JR, Lee HO, Pfeffer K *et al*. Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. *Nature* 2003; **425**: 968–973.
28 Wang X, Willenbring H, Akkari Y, Torimaru Y, Foster M, Al-Dhalimy M *et al*. Cell fusion is the principal source of bone-marrow derived hepatocytes. *Nature* 2003; **422**: 897–901.
29 Terada N, Hamazaki T, Oka M, Hoki M, Mastalerz DM, Nakano Y *et al*. Bone marrow cells adopt the phenotype of other cells by spontaneous fusion. *Nature* 2002; **416**: 542–545.
30 Idilman R, Erden E, Kuzu I, Ersoz S, Karasu Z, Karayalcin K *et al*. Recipient-derived hepatocytes in sex-mismatched liver allografts after liver transplantation: early versus late transplant biopsies. *Transplantation* 2004; **78**: 1647–1652.
31 Fogt F, Beyser KH, Poremba C, Zimmerman RL, Khettry U, Ruschoff J. Recipient-derived hepatocytes in liver transplants: a rare event in sex-mismatched transplants. *Hepatology* 2002; **36**: 173–176.
32 Wu T, Cieply K, Nalesnik MA, Randhawa PS, Sonzogni A, Bellamy C *et al*. Minimal evidence of transdifferentiation from recipient bone marrow to parenchymal cells in regenerating and long-surviving human allografts. *Am J Transplant* 2003; **3**: 1173–1181.
33 Kleeberger W, Rothamel T, Glockner S, Flemming P, Lehmann U, Kreipe H. High frequency of epithelial chimerism in liver transplants demonstrated by microdissection and STR-analysis. *Hepatology* 2002; **35**: 110–116.
34 Nelson JL, Furst DE, Maloney S, Gooley T, Evans PC, Smith A *et al*. Microchimerism and HLA-compatible relationships of pregnancy in scleroderma. *Lancet* 1998; **351**: 559–562.
35 Tanaka A, Lindor K, Gish R, Batts K, Shiratori Y, Omata M *et al*. Fetal microchimerism alone does not contribute to the induction of primary biliary cirrhosis. *Hepatology* 1999; **30**: 833–838.