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Effects of Bone Marrow and Hepatocyte Transplantation on Liver Injury

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Category: Transplantation/Immunology Seven Figures and Two Tables

ABSTRACT

Background. The therapeutic effects of bone marrow and hepatocyte transplantation were investigated regarding the treatment of retrorsine-partial hepatectomy-induced liver injury.

Methods. Analbuminemic F344alb rats were given two doses of retrorsine two weeks apart, followed four weeks later by transplantation with F344 rat bone marrow cells or hepatocytes immediately after a two-thirds hepatectomy. The survival rate, liver regeneration rate, liver functions, albumin-positive hepatocytes and normal albumin gene sequences in the liver and serum albumin levels were investigated in the recipients.

Results. Although 65% retrorsine/partial hepatectomy-treated F344alb died between one and 11 days after the partial hepatectomy, only 27.5% of the animals died following bone marrow transplantation, and 50% with hepatocyte transplantation. Both the bone marrow and hepatocyte transplantation ameliorated the acute liver injury after a partial hepatectomy. Bone marrow transplantation yielded a very small increase in the number of albumin-positive hepatocytes in the liver, while hepatocyte transplantation resulted in massive replacement of the liver tissues by the donor hepatocytes associated with an elevation of serum albumin after an extended time.

Conclusions. Both bone marrow and hepatocyte transplantation could prevent the acute hepatic injury, conceivably due to a paracrine mechanism.

Key words: Analbuminemic rats; Retrorsine; Partial hepatectomy; Hepatic injury; Bone marrow transplantation; Hepatocyte transplantation.

INTRODUCTION

Acute liver injury is still a major therapeutic challenge due to its high mortality rates as a result of multiorgan failure. Orthotropic allogenic liver transplantation is presently the ideal therapy for hepatic injury, but its application is limited by many problems, such as a shortage of donor organs, high cost, the requirement for life-long immunosuppression and the considerable risk accompanying this procedure [1]. Although the bioartificial livers have been devised for temporary liver support in acute liver injury, its clinical efficacy is still uncertain [2]. Cell transplantation therapy using isolated hepatocytes has been considered an attractive method, but the short life of isolated hepatocytes, limitations of the numbers of transplantable cell and problems associated with immunosuppression are serious obstacles to the establishment of this approach to the therapy [2-5].

Studies in humans [6, 7] and animals [8-12] suggested that bone marrow (BM) cells can differentiate into hepatocytes and repopulate within the liver under the special conditions such as whole body sublethal radiation and chronic hepatic injury. Although it is unclear whether the BM cells may increase the number of hepatocytes by transdifferentiation [9] or fuse with the host hepatocytes [13, 14], it is generally accepted that the BM cell transplantation (BM-Tx) can restore the liver following an injury. BM cells thus may be a facilitating source for transplantable cells in hepatic disease, because the patients' own BM cells are generally available.

Laconi *et al.* [15, 16] and Gordon *et al.* [17-19] established an animal model of hepatic injury that is induced by the administration of retrorsine (RS), a pyrrolizidine alkaloid, in combination with a two-thirds hepatectomy (PH). In this model, RS blocks the proliferating capacity of hepatocytes for a long period and thereby the liver regeneration is strongly suppressed, leading to hepatic injury [15-20]. When the hepatocytes are transplanted into the liver immediately after PH, more than 95% of the liver tissue is replaced by the donor hepatocytes within two to four months [15]. On the other hand, when BM cells were transplanted into the RS-PH-treated liver, only very few or no donor BM cell-derived hepatocytes are detected [21-24].

However, the therapeutic effect of BM-Tx and hepatocyte transplantation (HT-Tx) on RS-PH-induced hepatic injury has not yet been extensively studied.

Our previous studies have demonstrated that the transplantation model using Fischer 344 rats (F344) and F344 congenic Nagase's analbuminemic rats (F344alb) to be useful for the efficiency of repopulation of the hepatocytes after HT-Tx and BM-Tx in the liver [4, 25, 26]. F344alb have the genetic background of F344 and are otherwise normal except for a seven base pair deletion downstream of the exon H splice site within the ninth intron of albumin gene, which leads to the inability of hepatocytes to produce albumin [27]. In this model, the normal F344 cells can be transplanted within the liver of F344alb without the use of immunosuppressants and the transplanted cells are easily detectable using albumin as a marker.

In the present study, we investigated the therapeutic effect of BM-Tx and HT-Tx on RS-PH-induced hepatic injury using the F344/F344-alb transplantation model.

MATERIALS AND METHODS

Animals and Study Design

F344 were purchased from Charles River Japan (Yokohama, Japan) and F344alb were bred in the Asahikawa Medical College animal laboratory. All animals were maintained on daily cycles of alternating 12 hr light/darkness with food and water available *ad libitum*. All procedures performed on the animals were approved by the institutional committee according to the guidelines for humane care of laboratory animals. The F344alb were divided into five experimental groups (**Figure** 1): Group 1 (n=6), untreated control; Group 2 (n=5), PH alone; Group 3 (n=20), RS-PH with injection of the medium without cells; Group 4 (n=20), RS-PH with HT-Tx; and Group 5 (n=40), RS-PH with BM-Tx. In Group 5, the BM cells were transplanted into the portal vein in one group (Group 5a, n=20) and into the penile vein in other group (Group 5b, n=20). These animals were then sacrificed after 28 days after PH. For some rats, the blood samples were taken at the time of PH (0 day), 2, 7 and 14 days

after PH without sacrificing the animals.

RS-PH Treatment and Cell Transplantation

BM cells were isolated from the femurs of six weeks old male F344, centrifuged with Histopaque 1077 (Sigma-Aldrich, St Louis, MO) at 1,800 rpm for 30 min and kept in the modified Eagle medium solution (Sigma-Aldrich). Hepatocytes were isolated from eight weeks old male F344 using the two-step collagenase perfusion technique [28] and suspended in the Hanks' balanced salt solution (Sigma-Aldrich). Six to 10-months-old male F344alb were intraperitoneally administered two doses of RS (Sigma-Aldrich: 30 μ g/g body weight) two weeks apart and subjected to PH four weeks after the second RS treatment [15]. The RS-PH-treated F344alb were transplanted with 10⁷ F344 BM cells (cellular viability > 98%) via the portal or penile vein, or with 2 x 10⁶ F344 hepatocytes (cellular viability > 90%) via the portal vein.

Liver regeneration rate

The liver regeneration rate was calculated as follow; liver regeneration rate (%) = 100 x [C- (A-B)]/A, where A is the estimated liver weight before PH, B is the excised liver weight at the time of PH and C is the weight of the regenerated liver at the time of sacrifice [29].

Histology and immunohistochemistry

F344alb treated as above (Group 1-5) were sacrificed for histological analysis of the liver on day 2 and 28 after PH. The livers were perfusion-fixed with periodate-lysine-paraformaldehyde (PLP) solution via the portal vein and processed for histological and immunohistochemical analysis. Four slices were cut out from each hepatic lobe, further fixed in the PLP solution at 4°C overnight, dehydrated through gradient series of ethanol and embedded in paraffin. The tissues were cut into three µm-thick sections and stained with hematoxylin and eosin (HE). For immunohistochemistry, the slides were deparaffinized, incubated with 3% H₂O₂, reacted with 1/500-diluted rabbit anti-rat albumin antibody (Cappel, Malver, PA) and then incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG polymer (Dako, Carpinteria, CA). The antibody binding was visualized using the diaminobenzidine substrate-chromogen system (Dako, Carpinteria, CA), followed by counter-staining with hematoxylin. Single and double albumin positive (Alb+) hepatocytes and clusters consisting of larger numbers of Alb+ hepatocytes (3-100 cells and >100 cells) were counted microscopically and their numbers/cm² in the sections were determined for each animal using Scion Image software.

PCR detection of the albumin gene sequences

The PCR primers were designed to amplify a region including the seven base pairs lacking in the F344alb albumin gene [25, 26]. PCR was performed using the DNA isolated from the liver tissues, and the PCR products were separated by electrophorasis on 6% agarose gels, stained with ethidium bromide and visualized under UV illumination.

Measurement of serum albumin levels

The serum albumin levels were quantified using the enzyme-linked immunosorbent assay kits (SPI-BIO, Massy, France) before and 14 and 28 days after cell transplantation in each experimental group.

Liver Function Assays

Total bilirubin (TB), dissociative bilirubin (DB), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were quantified before and two and seven days after cells transplantation. TB and DB were quantified using the Vanadin acidic reaction (SRL, Japan), and AST and ALT were quantified by the Japan Society of Clinical Chemistry (JSCC, Japan)

Statistical Analysis

The data were statistically analyzed using the one-way ANOVA analysis of variance and the Chi-Square test (nonparametric) using the SPSS10.0 software. The survival rate was compared using a log rank analysis. Values are expressed as the mean \pm SD and *P* values less than 0.05 were considered significant.

RESULTS

Prolonged Survival of RS-PH-treated F344alb by BM-Tx and HT-Tx

Figure 2 shows that all the untreated animals (Group 1, n=6) and those treated with PH alone (Group 2, n=5) were alive until 28 days, but the fair number of RS-PH treated rats died between one and 11 days after PH. To statistically analyze the effect of cell transplantation, we used a larger number of animals for Group 3-5 than Group 1-2. Although 13 out of 20 RS-PH-treated F344alb (65%) in Group 3 died, 10 out of 20 (50%) in Group 4 (P=0.603, not significant in comparison to Group 3) and 11 out of 40 RS-PH-treated F344alb (27.5%) in Group 5 (P=0.006 in comparison with Group 3) died, following HT-Tx and BM-Tx, respectively. There was no significant difference in the death rate by BM-Tx via the portal vein (5 out of 20 animals died in Group 5a) and via the penile vein (6 out of 20 animals died in Group 5b: **Figure 2**).

Liver Regeneration Rate

The decreased death rate in the RS-PH-treated F344alb by BM-Tx and HT-Tx might be due to either the promotion of liver regeneration or the protection of the liver functions. The liver regeneration rate was 79.5 \pm 2.0% in the F344alb treated with PH alone (Group 2) at 28 days after PH, while it was 44.2 \pm 6.5% in the F344alb with RS/PH (Group 3: **Figure 3**), thus indicating that the RS treatment significantly suppressed liver regeneration. On the other hand, the liver regeneration rate was 62.9 \pm 9.7% in the F344alb with RS-PH plus HT-Tx (Group 4), while it was 49.1 \pm 10.2% in the F344alb with RS-PH plus BM-Tx (Group 5a), indicating that HT-Tx considerably improved the liver regeneration rate in the RS-PH-treated F344alb, while BM-Tx did not remarkably improve the liver regeneration rate. There was no significant difference in the bromodeoxyuridine labeling index in the livers at 24 hours after PH between the RS-PH treated (Group 3) and RS-PH plus BM-Tx treated F344alb (Group 5a), suggesting that BM-Tx did not promote proliferation of hepatocytes after PH in the RS-treated F344alb (data not shown).

Protection of Liver Functions by Cell Transplantation

Figure 4A shows the serum concentrations of TB, DB, ALT and AST to have slightly increased two days in F344alb with PH alone (Group 2), while they returned to the normal levels seven days after PH (data not shown). In contrast, the values were markedly elevated in the RS-PH-treated F344alb (Group 3) two days after PH, but the changes returned normal by seven days after PH (**Figure 4B**). In Group 3, the elevation of ALT values two days after PH was significantly higher in the F344alb that died until 7 days after PH than those that survived until 7 days after PH (*P* <0.001: **Figure 4B**). In the F344alb with RS-PH plus HT-Tx (Group 4), the values were much lower in comparison to Group 3 two days after PH and returned to the normal levels by seven days after PH (data not shown). In the F344alb with RS-PH plus BM-Tx (Group 5a), the values were almost in the normal ranges both two and seven days after PH (**Figure 4A** and data not shown). There were no significant difference in the serum levels of TNF- α and IL-10 in Group 3 and Group 5a two days after PH (data was not shown).

Histological Changes

The histological pattern of the liver of F344alb treated with PH alone (Group 2) was comparable to that of the normal F344alb 28 days after PH (**Figure 5A, B**). On the other hand, the liver of RS-PH-treated F344alb (Group 3) showed focal necrosis, edema in the Glisson's sheath and mild inflammatory infiltration two days after PH (**Figure 5C**), and the abnormally large-sized hepatocytes (megalocytosis) with frequent clusters of small-sized hepatocytes 28 days after PH (**Figure 5D**) as described previously [15, 17, 20]. The liver tissue of F344alb with RS-PH plus HT-Tx (Group 4) showed a much smaller degree of damage in comparison to the F344alb with RS-PH (Group 3) two days after PH (**Figure 5E**), and mainly consisted of normal-sized hepatocytes with occasional megalocytic hepatocytes 28 days after PH (**Figure 5F**). The liver histology was almost identical to that of the untreated F344alb two days in the F344alb with RS-PH plus BM-Tx (Group 5a: **Figure 5G**), but the histological pattern was not different from that of the F344alb with RS-PH

(Group 3) 28 days after PH (**Figure 5H**). Collectively, these findings indicate that either BM-Tx or HT-Tx prevented the histological damage that was observed shortly after PH in the RS-treated liver.

Alb+ hepatocytes in the F344alb Livers

The transplantation of F344 hepatocytes or BM cells into the liver of F344alb increased the number of F344-derived hepatocytes expressing normal albumin protein and mRNA [4, 25, 26]. To investigate whether the recovery of the liver volume and protection of the liver functions by cell transplantation to be correlated to the repopulation of the donor F344 hepatocytes or the donor F344 BM cell-derived hepatocytes in the liver, we measured the number of Alb+ hepatocytes in the liver of F344alb. A few Alb+ hepatocytes are detectable in untreated analbuminemic rats, which are increased in number with aging or carcinogen treatment [30]. The livers of the untreated F344alb (Group 1) contained very few Alb+ hepatocytes which were always present as single or pairs of cells 28 days after PH (Table 1). In addition, the livers of F344alb with PH alone (Group 2) and those with RS-PH treatment (Group 3) contained almost the same number of Alb+ hepatocytes (Figure 6A, B and Table 1), thus indicating that either PH or RS treatment did not increase the number of Alb+ hepatocytes. In contrast, the liver tissues were extensively replaced by large areas of Alb+ hepatocytes in F344alb with RS-PH plus HT-Tx (Group 4: Figure 6C and Table 1), which was more prominent 28 than 14 days after PH (data not shown). The liver in the F344alb with RS-PH plus BM-Tx (Group 5a) contained clusters of Alb+ hepatocytes, which were fewer in number and smaller in size than those observed in Group 4 (Figure 6D). There was no significant difference in the number of the Alb+ hepatocyte clusters after BM-Tx via the portal vein (Group 5a) and via the penile vein (Group 5b: Table 1). Thus, although HT-Tx led to extensive replacement of the liver tissues by Alb+ hepatocytes, BM-Tx did not.

Detection of the Normal Albumin Gene Sequences in the F344alb Liver

To validate that the Alb+ hepatocytes observed after cell transplantation were of

the donor F344 origin, the presence of the normal albumin gene sequences was investigated in the recipient livers. When PCR was performed for detection of the sequences spanning the seven base pair deletion in the ninth intron of the analbuminemic albumin gene, the 67 bp band was observed in the genomic DNA of the F344 rats, while the 60 bp band was observed for F344alb as described previously [4, 25, 26] (**Figure 7A, B**). PCR using the DNA isolated from the livers of Groups 1, 2, 3 and 5a generated only the 60 bp band, while the PCR generated both 60 bp and 67 bp bands for the DNA in the livers of F344alb with RS-PH plus HT-Tx (Group 4). On the other hand, when the F344alb with RS-PH plus BM-Tx (Group 5a) were sacrificed 1, 2, 3, 4 and 14 days after PH, although the 67 bp band was detected one and two days after BM-Tx, it was not detectable at the later time points (**Figure 7B**).

Elevation of Serum Albumin Levels

Lastly, the serum albumin levels in the F344alb were investigated, because the increased numbers of normal Alb+ hepatocytes in the liver might increase the serum albumin. Table 2 shows that the serum albumin levels were 3.3 ± 1.3 mg/dl in untreated F344alb (Group 1), while the values were 3,480.1 \pm 1,694.5 mg/dl in the The serum albumin levels in the F344alb with PH alone (Group 2) and those F344. with RS-PH (Group 3) were not different from the values of untreated F344alb before and 14 and 28 days after PH. In contrast, the serum albumin was markedly increased in the F344alb with RS-PH plus HT-Tx (Group 4) on day 14 and 28 after It is noticeable that the serum albumin levels in Group 4 overshot the average PH. of the normal F344 levels (4,449.7 \pm 1,863.0 vs 3,480.1 \pm 1,694.5 µg/dl) 28 days after PH. In the F344alb with RS-PH plus BM-Tx (Group 5a), there was no significant increase of serum albumin, conceivably due to the small number of Alb+ hepatocytes in the liver.

DISCUSSION

In the present study, we demonstrated that, although the RS-PH treatment led to

high mortality in F344alb due to severe hepatic injury shortly after PH, both BM-Tx and HT-Tx improved the survival rate of the RS-PH treated F344alb (The mortality was 27.5% vs 65% for the RS-PH plus BM-Tx and RS-PH groups, P=0.006, and 50% vs 65% for the RS-PH plus HT-Tx and RS-PH groups, P=0.603, statistically not significant) (**Figure 2**). We also demonstrated that the hepatic functions after PH were much improved in the RS-PH treated F344alb both by BM-Tx and HT-Tx (**Figure 4**), but this beneficial effect of cell transplantation did not depend on repopulation of hepatocytes derived from the transplanted cells (**Figures 6 and 7**). BM-Tx and HT-Tx, therefore, could be a new therapeutic approach for acute liver injury.

It is unlikely that the impaired hepatic functions in the RS-PH-treated F344alb were compensated by the transplanted cells themselves. In the case of HT-Tx, only 2×10^6 hepatocytes were transplanted (corresponding to 1/200 of the total recipient hepatocytes) into the liver and it took few weeks for the transplanted Alb+ hepatocytes to replace the hepatic tissues. In the case of BM-Tx, we demonstrated that only a small number of the BM cell-derived Alb+ hepatocytes were present in the liver 14 and 28 days after BM-Tx in our present and previous studies [25]. Several studies demonstrated that transplanted mesenchymal stem cells (MSC) or BM cells protected CCl₄-induced hepatic injury and promoted hepatic regeneration without their transdifferentiation into hepatocytes or fusion with the recipient hepatocytes [31-33]. A similar effect of MSC transplantation or BM-Tx has been reported with streptozotocin-induced pancreatic damage [34], bleomycin-induced lung damage [35] and ischemic cardiac injury [36]. In some cases, the mechanism of the protection was suggested to be increased vascularity by transdifferentiation of BM cells into vascular cells or secretion of angiogenic factors by BM cells [34-36]. Recently, it was further reported that MSC therapy prevented D-galactosamine or CCl₄-induced hepatic injury by the paracrine factor(s) generated by MSC [37, 38]. It is therefore conceivable that the beneficial effect of BM-Tx and HT-Tx on hepatic injury may be medicated by paracrine factor(s) generated by BM cells and hepatocytes, however further studies are needed to identify such factors.

It is unclear why BM-Tx more efficiently reduced the mortality of RS-PH treated F344alb than HT-Tx (**Figure 2**), despite the fact that both BM-Tx and HT-Tx protected the liver functions after PH (**Figure 5**). One possibility is that the putative paracrine factor(s) produced by BM cells may have more potent liver protective activity than those produced by hepatocytes. On the other hand, it is also possible that the BM cell-derived factor(s) may not only protect liver injury, but also protect the animals from other types of stress imposed by the RS-PH treatment such as vascular damage [39].

Braun *et al.* [40] reported the effect of HT-Tx on the transgenic mice expressing the herpes simplex virus thymidine kinase specifically in hepatocytes where the severity of liver injury can be controlled by changing the doses of ganciclovir. In their report, although HT-Tx could rescue mice with mild liver disease, it could not rescue the animals with severe liver disease, presumably due to the delayed repopulation of the transplanted hepatocytes. In the herpes simplex virus thymidine kinase-ganciclovir model, however, liver injury continued for a long period after ganciclovir treatment, in contrast to the case of RS-PH treatment where severe hepatic injury was temporal, and the animals could survive, if they could get past the critical period. It is, therefore, conceivable that repopulation of transplanted hepatocytes may be necessary for the restoration of the prolonged liver disease, while it may not be required for restoration of the acute phase of RS-PH-induced hepatic injury.

It was noticeable that the seven base deletion of the analbuminemic gene [27] was detectable only for two days after BM-Tx, thus indicating that majority of the transplanted BM cells did not reside in the liver beyond that time. Therefore, the therapeutic effect of BM-Tx on the RS-PH induced hepatic injury may not require the persistent engraftment of BM cells in the liver. It is also noteworthy that the survival rate of RS-PH-treated F344alb did not differ between BM-Tx via the portal vein (Group 5a) and the penile vein (Group 5b: **Figure 2**). However, whether the BM cells transplanted into the systemic circulation might have exerted a liver protective function without residing in the liver or after homing to the injured liver remains to be

determined.

According to clinical application of cell transplantation, Terai et al. [41] reported the liver functions to improve in the patients with liver cirrhosis after self-BM cell infusion. Fust et al. [42] showed that the combination of MSC transplantation with portal vein embolization in patients with large hepatic malignancies substantially increased hepatic regeneration in comparison to portal vein embolization alone. Our present study together with others (31-38) suggests that efficiency of the cell transplantation therapy for liver diseases may be due to the paracrine factors generated by the transplanted cells. Further investigation of the mechanisms of amelioration in hepatic injury by BM-Tx or HT-Tx could thus make it possible to develop an improved therapeutic regimen for acute hepatic injury.

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FIGURE LEGENDS

Figure 1. Experimental groups. Group 1; untreated F344alb, Group 2; PH alone, Group 3; two doses of RS (30 mg/Kg body weight) two weeks apart, and PH four weeks after the second RS treatment, followed by injection with the medium without cells. Group 4; RS-PH plus HT-Tx, Group 5; RS-PH plus BM-Tx via the portal vein (Group 5a) and via the penile vein (Group 5b). The animals were sacrificed 28 days after PH (X). For some rats, the blood samples were taken at the time of PH (0 day), 2, 7 and 14 days after PH (arrows).

Figure 2. Survival Curve. The survival rate is significantly lower in Group 3 in comparison to Group 1 and 2 (*:P = 0.014), while it is increased in Groups 4 and 5 (5a, 5b) as compared to Group 3 (Group 5 vs Group 3, P=0.006, and Group 4 vs Group 3, P=0.603, not significant). The survival rate did not differ with BM-Tx via the portal vein (Group 5a) or via the penile vein (Group 5b). **:P = 0.031, **:P = 0.021

Figure 3. Hepatic regeneration rate 28 days after PH. The hepatic regeneration rate is decreased in F344alb treated with RS-PH (Group 3) in comparison to those with HT alone (Group 2; P < 0.001), while it is significantly improved by HT-Tx (Group 4) (P = 0.001), but not by BM-Tx (Group 5a).

Figure 4. Liver functions tests. (A) The TB, DB, ALT and AST levels were slightly elevated in the F344alb treated with PH alone two days after PH (Group 2), while they were markedly increased in the group treated with RS-PH (Group 3). The levels were much lower in the groups with RS-PH plus HT-Tx (Group 4) and with RS-PH plus BM-Tx (Group 5a). (B) In Group 3, serum AST is increased two days after PH, while it returns to the basal level seven days after PH. Note that the values on day 2 were lower in the rats alive for seven days than in those dead until seven days after PH.

Figure 5. Liver histology. The livers of untreated F344alb (Group 1) (A), treated with PH alone (Group 2) (B), RS-PH (Group 3) (C, D), RS-PH plus

HT-Tx (Group 4) (E, F) and RS-PH plus BM-Tx (Group 5a) (G, H). Two days (C, E, G), and 28 days after PH (D, F, H). The areas surrounded by arrowheads (D, H) are clusters of small hepatocytes. Note that histological damage two days after PH in the F344alb with RS-PH (Group 3) (C) is improved by HT-Tx in Group 4 (E) and BM-Tx (Group 5a) (G). H & E staining. Magnification x200.

Figure 6. Albumin immunostaining of the F344alb livers 28 days after PH. Single Alb+ hepatocyte is seen in the liver of F344alb with PH alone (Group 2) (A) and treated with RS-PH (Group 3) (B). The liver tissue is extensively replaced by Alb+ hepatocytes in F344alb with RS-PH plus HT-Tx (Group 4) (C), but only few small Alb+ hepatocyte clusters were seen in the group with RS-PH plus BM-Tx (Group 5a).

Figure 7. The normal albumin gene sequences in the livers of F344alb. (A) The normal 67 bp band is generated by the PCR from the F344 DNA, while the aberrant 60 bp band is generated from the F344alb DNA (Group 1). Although only the 60 bp band is generated from the liver DNA of Groups 2, 3 and 5a, both 67 and 60 bp bands are generated from the liver DNA of F344alb with RS-PH plus HT-Tx (Group 4) 28 days after PH. (B) In F344alb with RS-PH plus BM-Tx (Group 5a), although both the 67 and 60 bp bands were seen one and two days after BM-Tx, the 67 bp band is no more detectable thereafter.

Table 1

Numbers of Alb+ Hepatocytes in the F344alb Livers 28 Days after Partial Hepatectomy

Group	Treatment			No.	Alb+ hepatoc	ytes/cm ² section		
	RS	PH	Тх	rats ^a	1 cell	2 cells	3-100 cells	> 100 cells
1	-	-	-	6/6	8.7 ± 0.7	2.5 ± 0.4	0	0
2	-	+	-	5/5	9.1 ± 0.9	2.8 ± 0.4	0.1 ± 0.1	0
3	+	+	-	3/7	8.5 ± 0.9	3.2 ± 0.5	0.1 ± 0.1	0
4	+	+	HT	8/10	8.1 ± 0.7	3.1 ± 0.5	$11.3 \pm 2.1*$	$13.4\pm2.8~^{\#}$
5a ^b	+	+	BMC	12/15	9.4 ± 0.4	3.4 ± 0.3	$14.6\pm1.6^*$	0
5b ^c	+	+	BMC	7/14	9.1 ± 0.4	3.2 ± 0.4	$14.4 \pm 1.8*$	0

^a Number of rats examined for immunohistochemical analysis on 28 days / Number of

survival rats 28 days

- ^b 5a; BMC-Tx via portal vein.
- ^c 5b; BMC-Tx via penile vein.
- * \boldsymbol{P} value < 0.05 in comparison with Groups 1 3.
- [#] \boldsymbol{P} value < 0.05 in comparison with Groups 1 3 and Group 5 (5a and 5b).

Table 2

Serum Albumin Levels in the F344alb and F344

Group	No nota ^a	Serum Albumin Levels (µg/dl)					
	no. rais –	Before PH	14 Days	28 Days			
F344	5 (5)	3480.1 ± 1694.5 *	nd	nd			
1	6 (6)	3.3 ± 1.3	nd	nd			
2	5 (5)	3.7 ± 1.5	3.1 ± 0.3	3.0 ± 0.1			
3	3 (10)	3.4 ± 1.2	2.3 ± 0.6	2.5 ± 0.2			
4	8 (17)	2.6 ± 0.5	$755.6 \pm 388.9^{\#}$	$4449.7 \pm 1863.0\ ^{\#}$			
5a	12 (16)	3.1 ± 1.0	2.9 ± 0.6	2.7 ± 1.1			

^a The left numbers; numbers of rats examined for serum albumin on 14 and 28 days, and the numbers in parenthesis; the numbers of rats examined for serum albumin before PH.

* \boldsymbol{P} value < 0.01 in comparison with Groups 1 – 5a.

[#] \boldsymbol{P} value < 0.01 in comparison with Groups 1 – 3 and 5a.

nd; not done

Group 1			<u> </u>
(n = 6)			ЪЦ
Group 2			<u>▼ X</u>
(n = 5)			
0	RS	RS	PH
Group 3 (n = 20)		V	▲A
x	RS	RS	PH HT-Tx
Group 4	\bigtriangledown	∇	▼ X
(n = 20)			
_	RS	RS	PH, BMC-Tx (portal)
Group 5a $(n - 20)$	V	\vee	▼ <u>X</u>
(11 = 20)			
Group 5b	RS ▽	RS ▽	PH, BMC-Tx (penile) ▼ X
(n = 20)			
	-42	-28	$0.2 \ 7 \ 14 \ 28 \ (days)$
	14	20	















