

## Brief Report

## TREATMENT OF THE CRIGLER-NAJJAR SYNDROME TYPE I WITH HEPATOCYTE TRANSPLANTATION

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**C**RIGLER-NAJJAR syndrome type I is a recessively inherited disorder characterized by severe unconjugated hyperbilirubinemia beginning at birth. The syndrome results from an absence of hepatic uridine diphosphoglucuronate (UDP) glucuronosyltransferase activity, which is essential for the conjugation and excretion of bilirubin. Because of the accumulation of unconjugated bilirubin in plasma, patients are at risk for kernicterus.<sup>1</sup> Although phototherapy successfully reduces serum bilirubin levels, patients are again at risk for kernicterus around the time of puberty, when phototherapy becomes less effective.<sup>2</sup> The necessary daily duration of phototherapy often approaches 14 to 16 hours. At present, liver transplantation is the only definitive treatment.<sup>3,4</sup>

Because hepatic architecture and function, except for bilirubin-UDP-glucuronosyltransferase activity, are normal in Crigler-Najjar syndrome type I, transplantation of isolated liver cells might be a safer and less invasive alternative treatment. Hepatocyte transplantation would not preclude future gene therapy or interfere with subsequent orthotopic liver transplantation, should it become necessary.<sup>5,6</sup>

The efficacy of hepatocyte transplantation in ameliorating pathologic processes in rodents<sup>7</sup> has prompted investigators to perform this procedure in a number of patients with acute liver failure and in one patient with ornithine transcarbamylase deficiency.<sup>8-12</sup> Thus far, however, clinically relevant long-term functioning of transplanted human hepatocytes

remains to be demonstrated. Because hepatocyte transplantation results in a long-term reduction in serum bilirubin concentrations in Gunn rats, the animal model of Crigler-Najjar syndrome type I,<sup>7,13</sup> and because hepatocyte transplantation in patients with this disorder would permit direct functional evaluation of the engrafted cells, we transplanted allogeneic hepatocytes into the liver of a patient with Crigler-Najjar syndrome type I. The hepatocytes were safely infused through the portal vein, survived for more than 11 months, and partially corrected the metabolic disorder.

## CASE REPORT

A 10-year-old girl had severe unconjugated hyperbilirubinemia at birth. Her serum bilirubin levels were reduced by phototherapy but were unaffected by phenobarbital. The clinical diagnosis of Crigler-Najjar syndrome type I was confirmed by a lack of bilirubin conjugates in the bile and by the presence of only traces of bilirubin-UDP-glucuronosyltransferase activity in a liver-biopsy specimen. In November 1994, an attack of streptococcal pharyngitis precipitated kernicterus, characterized by slurred speech, ataxia, and subsequent coma. The patient was treated with antibiotics, plasmapheresis, and intensive phototherapy and recovered without neurologic sequelae. After that time, she required 10 to 12 hours of phototherapy daily to maintain her serum bilirubin levels at 24 to 27 mg per deciliter (410 to 462  $\mu\text{mol}$  per liter).

The patient was placed on the liver-transplantation waiting list at the University of Nebraska Medical Center in April 1995. Investigations at the time revealed no evidence of hemolysis, liver disease other than the Crigler-Najjar syndrome, or residual neurologic damage. Once approval was obtained from the medical center's institutional review board and from the Food and Drug Administration (FDA) (under Investigational New Drug license 6880) to use hepatocyte transplantation to treat patients with life-threatening liver-based metabolic deficiencies, the hepatocyte-transplantation protocol was explained to the patient and her family, and they gave written informed consent for her participation.

Before transplantation, the patient's serum total bilirubin level ranged from 25.5 to 26.6 mg per deciliter (436 to 455  $\mu\text{mol}$  per liter). In preparation for hepatocyte transplantation, the phototherapy was intensified, and the patient was started on a regimen of calcium carbonate and tacrolimus (Prograf, Fujisawa, Deerfield, Ill.). After four days, the patient's bilirubin level fell to 18.1 mg per deciliter (310  $\mu\text{mol}$  per liter). Once a donor liver was obtained and the viability and quality of the hepatocytes were found to be acceptable, the patient was admitted to the hospital. With the patient under general anesthesia, a transcutaneous intrahepatic portal-vein catheter was placed for the infusion of hepatocytes, and a pulmonary-artery catheter was placed for monitoring.

Six hours later,  $7.5 \times 10^9$  hepatocytes were infused through the portal-vein catheter over a period of 15 hours. Portal-vein pressure was measured continuously during the infusion, and abdominal ultrasonography was performed to confirm the patency of the portal vein. The patient's oxygenation status and pulmonary-artery pressures were monitored continuously to assess whether there was substantial translocation of hepatocytes to the lungs. Methylprednisolone (1 g) was administered intravenously during the procedure, and intravenous corticosteroids continued to be administered during the next five days at a dose that was tapered from 200 mg per day to 20 mg per day. Oral prednisone was then given at a dose of 20 mg per day and tapered to 2.5 mg per day over the next six months. Tacrolimus was given orally, and the dose was adjusted to maintain serum levels of 10 to 15 ng per milliliter.

Three hours after the completion of the hepatocyte infusion, the portal-vein catheter was removed at the bedside. The patient was discharged 20 hours later.

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## METHODS

### The Hepatocyte Donor

Hepatocytes were recovered post mortem from a five-year-old boy whose liver could not be placed for organ transplantation because there were no appropriate recipients compatible with this donor with respect to size and blood group on the waiting list of the United Network for Organ Sharing. The liver enzymes were normal, and the liver had no traumatic damage. The donor liver was flushed with University of Wisconsin preservation solution.<sup>14</sup> A biopsy showed no histologic evidence of macrovesicular fat. Serologic tests were negative for hepatitis B and C, human immunodeficiency virus, and cytomegalovirus. Titers of antibodies to Epstein-Barr virus indicated previous exposure to this virus. The hepatocyte donor and recipient were matched only for ABO blood-group compatibility.

### Isolation and Processing of Hepatocytes

Hepatocytes were isolated at the University of Pittsburgh by three-step collagenase perfusion, as described by Strom et al.<sup>15</sup> The ratio of hepatocytes to nonparenchymal cells was increased by three consecutive centrifugation steps; next, the cells were resuspended in University of Wisconsin solution at 4°C at a concentration of 5 million cells per milliliter. Cells were then transferred by air-courier service to the University of Nebraska Medical Center. Transport took approximately 5 hours; the first cell infusion was begun 6.5 hours after the cells were isolated. The cell suspension was tested for mycoplasma, endotoxin, bacteria, and fungus as required by the FDA. Before infusion, aliquots of  $1 \times 10^9$  to  $1.5 \times 10^9$  hepatocytes were removed from the suspension, washed, and resuspended in 75 ml of cold lactated Ringer's solution in 600-ml polyvinyl chloride bags (Terumo, Tokyo, Japan). The remainder of the cell suspension was stored for 1 to 15 hours at 4°C in University of Wisconsin solution.

### Viability and Function of Hepatocytes

Morphologically, more than 95 percent of the cells were hepatocytes. The initial rate of viability was approximately 90 percent, as assessed by cell-membrane exclusion of trypan blue dye. Plating efficiency was determined to evaluate cell quality further. Approximately 50 percent of donor hepatocytes adhered to tissue-culture plates in hormonally defined serum-free medium<sup>16</sup>; viability and plating efficiency did not deteriorate over the subsequent 24 hours. Donor hepatocytes were homozygous for a normal TATAA element with six TA repeats (A(TA)<sub>6</sub>TAA) upstream from the first exon of the bilirubin-UDP-glucuronosyltransferase-1 gene.<sup>17</sup> If the donor gene had had a variant of this promoter region, found in Gilbert's syndrome, the effectiveness of enzyme replacement by hepatocyte transplantation would have been reduced. At a bilirubin concentration of 80 μM in culture medium, the donor hepatocytes produced bilirubin glucuronides at the rate of 3.5 nmol per microgram of protein per hour, of which 80 percent was bilirubin diglucuronide. Glucuronosyltransferase activity toward bilirubin in digitonin-activated cell homogenates at a bilirubin concentration of 80 μM was similar to that in human liver homogenates.<sup>18</sup>

### Transplantation of Hepatocytes

The left portal vein was punctured percutaneously with a 21-gauge needle, and access was obtained with a micropuncture introducer set (Cook, Bloomington, Ind.), under ultrasound guidance. A 5-French Kumpe catheter (Cook) was manipulated into the main portal vein and, after the position of the catheter was confirmed by the injection of contrast material, the catheter was sutured to the anterior abdominal wall and the hepatocytes were infused.

The liver of a 70-kg adult is estimated to contain approximately  $2.8 \times 10^{11}$  hepatocytes, or  $4 \times 10^9$  cells per kilogram of body weight.<sup>19</sup> In an attempt to achieve 2.5 percent reconstitution of the liver with transplanted cells, hepatocytes equivalent to approx-

imately 5 percent of the normal host hepatocyte mass were infused, with the expectation that approximately 50 percent of the cells would engraft. Cells were intermittently agitated to avoid clumping and were infused by means of a pump ( $1 \times 10^9$  to  $1.5 \times 10^9$  cells over a period of 30 minutes) in three separate infusions separated by 4 to 6 hours.

Engraftment and function of the transplanted hepatocytes were evaluated by high-performance liquid chromatographic analysis of pigments in bile samples and by measurement of serum bilirubin levels one to three times per week. Liver biopsies were performed at the time a transhepatic portal catheter was placed and again seven days after transplantation, to measure enzyme activity and to assess any possible damage to the liver.

### Measurement of Bilirubin-UDP-Glucuronosyltransferase Activity and Bilirubin Conjugates

To measure glucuronosyltransferase activity, a homogenate of 20 percent of the biopsy specimen was prepared in 0.25 M sucrose and 10 mM TRIS-hydrogen chloride (pH 7.4) and assayed as previously described.<sup>18</sup> Bile samples were collected through a nasal-duodenal tube, with or without the intravenous administration of octapeptide cholecystokinin, protected from light, and stored at -80°C. Bile pigments were analyzed by high-performance liquid chromatography.<sup>18</sup>

## RESULTS

### Hemodynamic and Biochemical Response to the Intraportal Infusion of Hepatocytes

The patient had been extubated and was awake during the hepatocyte infusion and had no notable changes in blood pressure, pulse rate, temperature, pulmonary-artery pressure, central venous pressure, pulmonary-artery wedge pressure, or oxygen saturation. The portal-vein pressure did not increase more than 4 mm Hg for more than five minutes. Abdominal ultrasonography revealed no formation of clots or changes in flow in the portal vein.

The hemoglobin level, platelet count, serum creatinine level, coagulation profile, and serum alkaline phosphatase and γ-glutamyltransferase activity remained within normal ranges. Serum aspartate and alanine aminotransferase activity before the infusion was 35 IU per liter and 47 IU per liter, respectively, and reached maximal levels of 269 IU per liter and 131 IU per liter during the final infusion. Aspartate and alanine aminotransferase activity returned to base-line values 38 hours and five days, respectively, after the final infusion.

### Changes in the Serum Bilirubin Level and Requirement for Phototherapy

The serum total bilirubin level fell to 13.2 mg per deciliter (226 μmol per liter) with hydration and continuous high-intensity phototherapy in the hospital. The day after the hepatocyte infusion, phototherapy was reduced to pretransplantation levels. The serum bilirubin level increased to 21.5 mg per deciliter (368 μmol per liter) on day 7, and phenobarbital was started in an attempt to induce glucuronosyltransferase activity in the transplanted normal hepatocytes. After a liver biopsy on day 7, there was a further increase in the serum bilirubin level, which peaked at 26.1 mg per deciliter (446 μmol per liter)

on day 11. Thereafter, the serum bilirubin level progressively declined, although there was considerable fluctuation during the first three months (Fig. 1). By day 35, the duration of phototherapy had been reduced to six to eight hours per day. Bilirubin levels stabilized at 10.6 to 14.0 mg per deciliter (181 to 239  $\mu\text{mol}$  per liter) until bacterial sinusitis developed approximately six months after transplantation. During the infection, the total bilirubin level increased to 18.0 mg per deciliter (308  $\mu\text{mol}$  per liter), but it returned to previous levels after two months of oral antibiotic therapy.

Bile pigments extracted from the patient's serum showed no detectable bilirubin glucuronides before cell transplantation. After transplantation, 0.5 to 1 percent of serum bilirubin was made up of bilirubin glucuronides, predominantly bilirubin monoglucuronide. As of April 1, 1998, 11 months after the transplantation, the patient had a serum total bilirubin level of approximately 14 mg per deciliter (240  $\mu\text{mol}$  per liter) and was receiving only six to seven hours of phototherapy daily.

#### Analysis of Bile

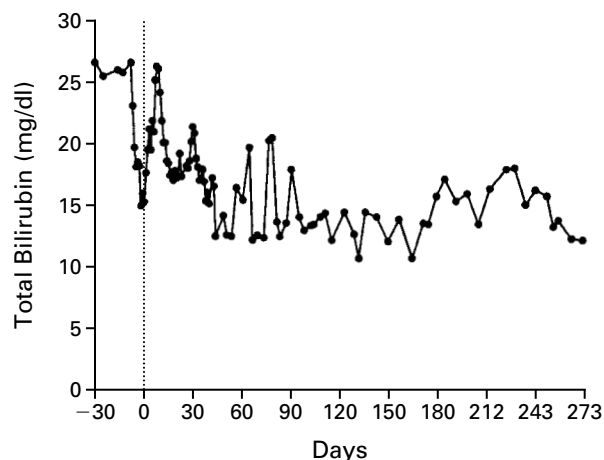
Bile pigments were analyzed in samples collected the day before transplantation and 1, 8, 14, and 180 days thereafter. Pretransplantation bile contained predominantly unconjugated bilirubin, with a trace of bilirubin monoglucuronide. After transplantation, all bile samples showed bilirubin diglucuronide and monoglucuronide, along with unconjugated bilirubin. The relative amounts of the three types of bilirubin were determined by the integration of the areas under the chromatography peaks (Fig. 2). Of the pigments excreted in the bile, 33 percent were bilirubin glucuronides. Interestingly, about 80 percent of the conjugates consisted of bilirubin diglucuronide.

#### Liver Biopsy and Bilirubin-UDP-Glucuronosyltransferase Activity

Histologically, there was no change in the liver architecture or other evidence of liver damage in the liver biopsies. Before transplantation, the hepatic enzyme activity was 8 pmol per milligram of protein per hour, which is approximately 0.4 percent of mean ( $\pm\text{SE}$ ) normal hepatic bilirubin-UDP-glucuronosyltransferase activity toward bilirubin ( $2000 \pm 800$  pmol per milligram of protein per hour).<sup>18</sup> Seven days after transplantation, this activity was 110 pmol per milligram of protein per hour (approximately 5.5 percent of mean normal enzyme activity).

### DISCUSSION

In the patient we treated, isolated hepatocytes, representing a substantial fraction of the liver mass, could be safely infused through the portal vein for engraftment in the liver and survived for more than 11 months, partially correcting a liver-based metabolic

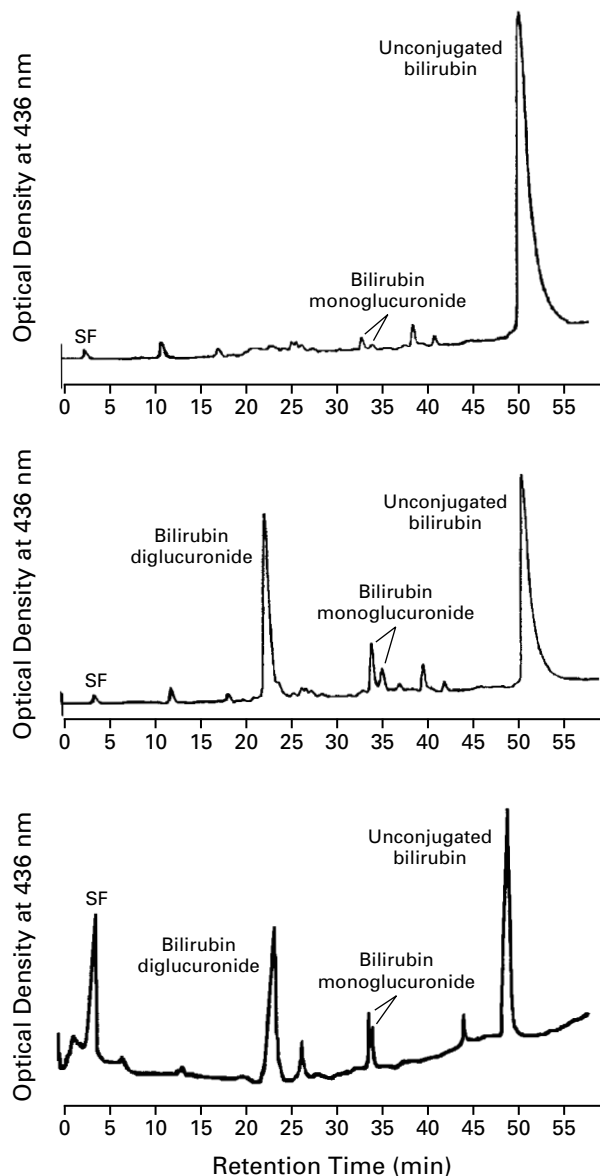


**Figure 1.** Changes in Serum Total Bilirubin Levels after the Transplantation of Hepatocytes.

Before transplantation, which occurred on day 0, the patient was treated with hydration and continuous high-intensity phototherapy to decrease the likelihood that hyperbilirubinemia and kernicterus would result from the stress of the hepatocyte infusion. The day after the hepatocyte infusion, phototherapy was reduced to pretransplantation levels. Bile specimens were collected with use of intravenous octapeptide cholecystokinin on days 1, 8, 14, and 180 after transplantation. The patient was given phenobarbital in an attempt to induce enzyme activity in the transplanted normal hepatocytes on day 7. A liver biopsy was performed on day 7, and the duration of phototherapy was reduced to six to eight hours per day by day 35. Bacterial sinusitis developed and lasted approximately from day 190 to day 245. To convert values for bilirubin to micromoles per liter, multiply by 17.1.

disorder. The presence of bilirubin-UDP-glucuronosyltransferase activity in the liver and bilirubin conjugates in bile provides evidence of hepatocyte engraftment. Bilirubin diglucuronide was the predominant conjugate excreted in the bile, paralleling the pattern in bile specimens from normal subjects and differing from that in specimens from patients with partial enzyme-deficiency states, such as Crigler-Najjar syndrome type II and Gilbert syndrome.<sup>20</sup> Thus, the engrafted cells, which have a normal complement of the enzyme, appear to secrete bilirubin glucuronides in the proportions found in normal bile.

In previous studies of human hepatocyte transplantation for acute liver failure or liver-based metabolic disorders, a small number of transplanted cells were identified at the transplant site days to weeks after transplantation. Because the patients were not randomly assigned to treatment groups and often underwent subsequent whole-liver transplantation, whether the transplanted cells function in a clinically significant way has been difficult to assess. In contrast, we evaluated the function of the engrafted hepatocytes by chromatographic analysis of bile pigments. Although possible sampling errors preclude precise quantification, our finding that hepatic bilirubin-



**Figure 2.** High-Performance Liquid Chromatographic Analysis of Bile Collected before the Transplantation of Hepatocytes (Top Panel) and 14 and 180 Days after Transplantation (Middle and Bottom Panels, Respectively).

The bile obtained before transplantation contained predominantly unconjugated bilirubin, with only a trace of bilirubin monoglucuronide — a pattern often seen in patients with Crigler–Najjar syndrome type I. After transplantation, the bile samples contained bilirubin diglucuronide and bilirubin monoglucuronide in addition to unconjugated bilirubin. The relatively high level of unconjugated bilirubin in the bile is probably the result of phototherapy. Injection of prepared biosamples occurred at time 0. SF denotes solvent front and indicates the time at which unretained molecules are released from the column.

UDP-glucuronosyltransferase activity after transplantation was approximately 5 percent of normal suggests that a majority of the transplanted cells were engrafted. The longevity of the cells transplanted into human livers remains to be established; however, when hepatocytes have been transplanted into the livers of rodents, they are rapidly integrated into the liver cords, with lifelong survival and function.<sup>21</sup>

Of the 19 patients with Crigler–Najjar syndrome type I listed in the world registry who have undergone liver transplantation, 3 have required a second transplantation and 2 have died.<sup>22</sup> The lower surgical risk and the reduced consequences of graft loss associated with hepatocyte transplantation, as opposed to liver transplantation, could benefit patients with this disease. The chief potential complications of intraportal hepatocyte infusion include portal-vein thrombosis with concomitant liver injury, portal hypertension, hemorrhage, and passage of cells to the lungs, with resulting pulmonary embolism. None of these adverse events occurred in our patient, and in contrast to findings in large animals,<sup>23</sup> her serum aminotransferase activity increased only 200 to 700 percent (probably as a result of transient ischemia of the native hepatocytes) and returned to normal within hours or days. Because the patient has not had opportunistic infections, the consequences of immunosuppression have been minimal. Furthermore, rejection has not been a problem to date. It remains to be determined whether patients can tolerate a more rapid reduction in the degree of immunosuppression, and possibly even its withdrawal, which would further reduce the long-term risk of the hepatocyte-transplantation procedure.

On the basis of experience with patients with Crigler–Najjar syndrome type II, the current serum bilirubin levels in our patient are unlikely to cause bilirubin toxicity. In fact, the patient was able to recover from a bacterial sinus infection without hospitalization or an increase in the duration of her phototherapy, when in the past such an infection might have required treatment in the intensive care unit. To make it easier to establish the duration of graft survival, we have not transplanted additional hepatocytes. However, because of the relatively low technical risk and cost of the procedure, multiple hepatocyte infusions could be contemplated as a way to engraft enough hepatocytes to eliminate any need for phototherapy. It is not known, however, whether multiple infusions would increase the risk of rejection.

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## REFERENCES

1. Crigler JF Jr, Najjar VA. Congenital familial nonhemolytic jaundice with kernicterus. *Pediatrics* 1952;10:169-79.
2. Pett S, Mowat AP. Crigler-Najjar syndromes type I and II: clinical experience — King's College Hospital 1972-1978: phenobarbitone, phototherapy and liver transplantation. *Mol Aspects Med* 1987;9:473-82.
3. Kaufman SS, Wood RP, Shaw BW Jr, et al. Orthotopic liver transplantation for type I Crigler-Najjar syndrome. *Hepatology* 1986;6:1259-62.
4. Whittington PF, Emond JC, Heffron T, Thistlethwaite JR. Orthotopic auxiliary liver transplantation for Crigler-Najjar syndrome type I. *Lancet* 1993;342:779-80.
5. Grossman M, Raper SE, Kozarsky K, et al. Successful ex vivo gene therapy directed to liver in a patient with familial hypercholesterolaemia. *Nat Genet* 1994;6:335-41.
6. Fox IJ, Chowdhury NR, Gupta S, et al. Conditional immortalization of Gunn rat hepatocytes: an ex vivo model for evaluating methods for bilirubin-UDP-glucuronosyltransferase gene transfer. *Hepatology* 1995;21:837-46.
7. Fox IJ, Chowdhury NR, Chowdhury JR. Hepatocyte transplantation in liver failure and inherited metabolic disorders. In: Lee WM, Williams R, eds. *Acute liver failure*. Cambridge, England: Cambridge University Press, 1997:285-99.
8. Strom SC, Fisher RA, Thompson MT, et al. Hepatocyte transplantation as a bridge to orthotopic liver transplantation in terminal liver failure. *Transplantation* 1997;63:559-69.
9. Habibullah CM, Syed IH, Qamar A, Taher-Uz Z. Human fetal hepato-

- cyte transplantation in patients with fulminant hepatic failure. *Transplantation* 1994;58:951-2.
10. Bilir B, Durham JD, Krystal J, et al. Transjugular intra-portal transplantation of cryopreserved human hepatocytes in a patient with acute liver failure. *Hepatology* 1996;24:Suppl:308A. abstract.
11. Mito M, Kusano M. Hepatocyte transplantation in man. *Cell Transplant* 1993;2:65-74.
12. Reyes J, Rubenstein WS, Miele L, et al. The use of cultured hepatocyte infusion via the portal vein for the treatment of ornithine transcarbamoylase deficiency by transplantation of enzymatically competent ABO/Rh-matched cells. *Hepatology* 1996;24:Suppl:308A. abstract.
13. Gunn CH. Hereditary acholuric jaundice in a new mutant strain of rats. *J Hered* 1938;29:137-9.
14. Belzer FO, Southard JH. Principles of solid-organ preservation by cold storage. *Transplantation* 1988;45:673-6.
15. Strom SC, Pisarov LA, Dorko K, Thompson MT, Schuetz JD, Schuetz EG. Use of human hepatocytes to study P450 gene induction. *Methods Enzymol* 1996;272:388-401.
16. Block GD, Locker J, Bowen WC, et al. Population expansion, clonal growth, and specific differentiation patterns in primary cultures of hepatocytes induced by HGF/SF, EGF and TGF alpha in a chemically defined (HGM) medium. *J Cell Biol* 1996;132:1133-49.
17. Bosma PJ, Chowdhury JR, Bakker C, et al. The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. *N Engl J Med* 1995;333:1171-5.
18. Chowdhury JR, Chowdhury NR, Wu G, Shouval R, Arias IM. Bilirubin mono- and diglucuronide formation by human liver in vitro: assay by high-pressure liquid chromatography. *Hepatology* 1981;1:622-7.
19. Sussman NL, Kelly JH. Artificial liver: a forthcoming attraction. *Hepatology* 1993;17:1163-8.
20. Fevery J, Blanckaert N, Heirwegh KPM, Preaux A-M, Berthelot P. Unconjugated bilirubin and an increased proportion of bilirubin monoconjugates in the bile of patients with Gilbert's syndrome and Crigler-Najjar disease. *J Clin Invest* 1977;60:970-9.
21. Gupta S, Rajvanshi P, Lee CD. Integration of transplanted hepatocytes into host liver plates demonstrated with dipeptidyl peptidase IV-deficient rats. *Proc Natl Acad Sci U S A* 1995;92:5860-4.
22. van der Veere CN, Sinaasappel M, McDonagh AF, et al. Current therapy for Crigler-Najjar syndrome type I: report of a world registry. *Hepatology* 1996;24:311-5.
23. Benedetti E, Kirby JP, Asolati M, et al. Intrasplenic hepatocyte allotransplantation in Dalmatian dogs with and without cyclosporine immunosuppression. *Transplantation* 1997;63:1206-9.

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