Liver Cell Transplantation – Novel Animal Model for Human Hepatic Viral Infections

Catherine H. Wu, Edwin C. Ouyang, Cherie Walton, George Y. Wu

Department of Medicine, Division of Gastroenterology-Hepatology, University of Connecticut Health Center, Farmington, Conn, USA

Aim. To generate a model of human hepatitis B infection (HBV) in immunocompetent rats with chimeric human liver.

Results. Normal rats were tolerized to human hepatocytes by exposure to human hepatocytes at day 17 of gestation. We transplanted human hepatocytes and inoculated HBV into the rats after birth. Mixed lymphocytes assay, a measure for tolerance, indicated that animals fetally exposed to human hepatocytes developed tolerance to human hepatocytes. Spleen lymphocytes from tolerized animals did not proliferate when challenged with donor human hepatocytes. In contrast, control animals given saline fetally developed no tolerance to human hepatocytes. Tolerant animals with transplanted human hepatocytes were susceptible to HBV infection. Western blot analysis and immuno-histochemistry of liver sections from tolerized, HBV infected animals with transplanted human hepatocytes showed the presence of functioning human hepatocytes that synthesized human albumin, of which 30% were also positive for HB surface antigen and HBV DNA. The presence of covalent closed circular HBV DNA in the liver indicated active HBV viral replication.

Conclusion. Tolerized rats with chimeric human livers can be infected with HBV and used as an animal model for HBV infection. Tolerized rats with chimeric human livers can also be used for generating models of other human hepatic viral diseases.

Key words: cell transplantation; hepatitis B surface antigens; hepatitis B virus; liver; models, animal; transplantation tolerance

The study of many human diseases is hampered by the lack of useful animal models. This is especially true for viral infections of the liver that result in acute and chronic hepatitis. Persistent hepatitis B (HBV) (1) and chronic hepatitis C (HCV) (2) viral infections are major health problems worldwide. HBV and HCV are highly tissue-(liver) and species-selective. HBV animal models based on homologous viruses in non-primates, such as woodchuck (3), or HBV in immunodeficient (4) or transgenic animals (5), while providing useful information lack many aspects of human disease. Beside the chimpanzee, there are no animal models for HCV (6). Research on transgenic animals expressing individual HCV gene products has yielded conflicting results where the function of the proteins is concerned. Until there is an experimental model system that supports HCV replication and infection, the role of each individual protein in the pathogenic outcome of HCV infection can not be clearly delineated.

In recent years, transplantation of normal or genetically altered syngeneic and allogeneic hepatocytes has been used successfully for gene therapy of different diseases in animal models (7,8) and humans (9,10). Hepatocyte transplantation experiments have also provided useful information on the localization and vascular and tissue requirements for the maintenance of transplanted cells (11), liver gene regulation (12), and regulation of liver growth. In most allogeneic transplantation experiments, immunosuppression has been necessary to sustain functioning transplanted cells. In developing animal models for human HBV or HCV viral diseases, it is important that they have intact and normal immune system because the host immune system plays an important role in the pathogenesis of the viral infections (13). Our hypothesis was that tolerizing rats with immature immune system to human hepatocytes would result in rats that can host transplanted human hepatocytes. Tolerant rats with chimeric human livers could be infected with HBV or HCV. Here we review our data that supported this hypothesis.

Generation of Chimeric Human Liver in Normal Rats

Induction of Tolerance to Human Hepatocytes

The major obstacle in establishing chimeric human liver in a normal rat is the rejection of transplanted xenogenic human cells by the host immune system. Therefore, our first step was to induce donor-specific tolerance towards human cells in the host before the transplantation.
Earlier, Medawar and co-workers (14) showed that "actively acquired tolerance" to foreign cells could be achieved by exposing fetal animals to the foreign cell. This method of tolerization was used by Kline et al (15) for significant prolongation of cardiac allograft survival. To induce tolerance, human hepatocytes were injected into intraperitoneal cavity of fetal rats at 15-17 days of gestation, a time frame when T-cells are educated to distinguish self from foreign antigens. Mixed lymphocyte assay, a measure of tolerance, indicated that spleen lymphocytes from control saline-treated animals were stimulated to proliferate when mixed with human hepatocytes, as indicated by the increased \(^{3}\text{H}\)-thymidine uptake (Fig. 1, column 3). In contrast, lymphocytes from the animals that were fetally exposed to human hepatocytes were not stimulated to proliferate when mixed with donor human hepatocytes (Fig. 1, column 4). This indicated that the animals became tolerant to human hepatocytes. Similarly, lymphocytes from the animals that were fetally exposed to and subsequently transplanted with human hepatocytes were not stimulated to proliferate when mixed with donor human hepatocytes (Fig. 1, column 5). The degree of \(^{3}\text{H}\)-thymidine uptake in lymphocytes from fetally tolerized rats when exposed to donor human hepatocytes was similar to that found in lymphocytes alone (Fig. 1, column 1) or in human hepatocytes alone (Fig. 1, column 2).

In animals, intrathymic injection of the donor cells or antigens (16) or oral gavaging of large concentrations of foreign antigen (17) can also induce tolerance to foreign antigen and donor cells. We tested which of the three methods would be optimal in inducing tolerance to human hepatocytes in the normal rat (Fig. 2). Although intrathymic injection (Fig. 2, column 3) and oral ingestion (Fig. 2, column 4) can induce tolerance, exposure of fetal rats to human hepatocytes (Fig. 2, column 2) produced the greatest degree of tolerance. Thus, rats with normal immune system can be made tolerant to human hepatocytes by fetal exposure to the human cells.

**Transplantation of Human Hepatocytes into Tolerized Rats**

Can rats tolerant to human hepatocytes be successful hosts to transplanted human hepatocytes?

To answer this question, human hepatocytes were transplanted to rats fetally tolerized with human hepatocytes at 17 day of gestation within 24 h after their birth. To determine the success of human hepatocyte transplantation, serum and liver samples were collected. Western blot analysis using an affinity purified anti-human albumin antibody indicated that human albumin could be detected in the serum of tolerized rats that received transplanted hepatocytes (Fig. 3). The antibody was specific for human albumin (Fig. 3, column 1) and could not detect standard rat albumin (Fig. 3, column 2). In tolerized rats that received transplanted column hepatocytes, human albumin could be detected in serum at least up to 6 weeks after transplantation (Fig. 3, columns 3-7). Rats tolerized and transplanted with a human fibroblast cell line, IMR 90, did not produce human serum albumin (Fig. 3, column 8), which indicated that albumin production is specific to human liver cells only.

Human albumin production by transplanted human liver cells was also confirmed by immunofluorescence of fresh frozen liver tissues. Figure 4 is a representative immunofluorescence of liver section taken from rat 3 weeks after the transplantation. In control animals, which fetally received injection of saline and...
were not transplanted with human hepatocytes, no
immunofluorescence for human albumin could be
detected (Fig. 4, panel A). Three weeks after human
hepatocytes transplantation, tolerized rat was posi-
tive for human albumin, as indicated by the positive
fluorescent cells (Fig. 4, panel B), whereas tolerized
control animals were not positive for human albumin
(Fig. 4, panel C).

**Generation of a Rat Model of HBV Infection
in Tolerized Rats with Chimeric Human Liver**

To generate HBV infection, tolerized rats to which
human hepatocytes were transplanted 24 h after birth
were inoculated with HBV virus one week after the
transplantation. Serum samples were collected
weekly. Serial liver biopsies were performed at timed
intervals after infection. Immuno-histochemical anal-
ysis (Fig. 5) revealed that human hepatocytes contain-
ing human albumin and producing HBV surface anti-
gen were present in rat livers at 15 weeks after HBV
infection (Fig. 5, panels A and B). In addition, 30% of
the cells positive for human albumin were also posi-
tive for HBV surface antigen. Livers from tolerized an-
imals that received transplanted hepatocytes but had
no HBV infection were positive for human albumin
(Fig. 5, panel C), but did not have HBV surface anti-
gen staining (Fig. 5, panel D). When inoculated with
HBV, these tolerized animals were negative for both
human albumin and HBV surface antigen (Fig. 5, pan-
els E and F). To determine that HBV surface antigen
in the human liver cells did indeed come from viruses,
the presence of HBV viral DNA (HBV DNA) was de-
tected by *in situ* hybridization with a digoxigenin-
HBV DNA probe (Fig. 6). HBV DNA positive cells
were present only in the liver from tolerized animals
that received transplanted hepatocytes and were in-
fected with HBV (Fig. 6, panel A). Animals that did
not receive transplanted human hepatocytes but were
infected with HBV were not positive for HBV-DNA
(Fig. 6, panel C). Similarly, livers from tolerized con-
trol animals (Fig. 6, panel D) and animals tolerized

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**Figure 3.** Western blot of rat serum, developed with anti-hu-
man albumin antibody and horseradish peroxidase-labeled
secondary antibody. Lane 1: 10 ng standard human albumin.
Lane 2: 10 ng standard rat albumin. Lane 3-7: serum from
tolerized rat with transplanted human hepatocytes. Lane 3:
two days after transplantation. Lane 4: two weeks after trans-
plantation. Lane 5: three weeks after transplantation. Lane 6:
five weeks after transplantation. Lane 7: six weeks after trans-
plantation. Lane B: serum from a rat tolerized and transplant-
ed with human fibroblast cell line IMR-90.

**Figure 4.** Immunofluorescence of fresh frozen liver tissue
using anti-human antibody and Texas-red labeled second-
ary antibody. Panel A: liver from control fetally treated rat
without transplanted human hepatocytes. Panel B: liver
from fetally tolerized rat 3 weeks after human hepatocytes
transplantation. Panel C: liver from fetally tolerized rat
without transplanted cells. Panel D: same as panel B, but
without secondary antibody.

**Figure 5.** Immunofluorescence of fresh frozen liver tissue
using anti-human antibody developed with Texas-red la-
beled secondary antibody and anti-HBV surface antigen de-
veloped with FITC-labeled secondary antibody. Panels A and
B: liver from tolerized rat with transplanted human hepato-
cytes, and infected with HBV at 15 weeks after infec-
tion. Panels C and D: liver from tolerized rat with trans-
planted human hepatocytes but without HBV infection.
Panels E and F: liver from tolerized rat without transplanted
human hepatocytes but inoculated with HBV.
with human liver cell transplantation (Fig. 6, panel B) were negative for HBV DNA. If infection had taken place and viruses were replicating, DNA from the viruses should be present in sera of infected animals. Serum HBV DNA was confirmed by the presence of an expected 355-bp fragment of HBV genome spanning nt 2079-2434 (Fig. 7). Serum HBV DNA was detectable at 1 week after the infection (Fig. 7, lanes 4 and 7) and remained detectable over 15 weeks (the duration of the experiment) after HBV inoculation (Fig. 7, lanes 6 and 9). Serum HBV DNA was not detected in tolerized rats that received transplanted hepatocytes and were not inoculated with HBV (Fig. 7, lanes 10 and 11), nor was it found in tolerized rats that did not receive transplanted hepatocytes but were inoculated with HBV (lanes 12-17). At 15 weeks after HBV infection, there were 5,000 copies of HBV genome/mL serum in tolerized rats transplanted with human liver cells and inoculated with HBV. Finally, HBV replication could be demonstrated in livers by the presence of covalent closed circular HBV DNA (Fig. 8). It was detected by polymerase chain reaction primers specific to the region of HBV genome that is incomplete in the plus strand of the viral particle, but is covalently closed during HBV replication (Fig. 8, lanes 4 and 5). The same 698-bp band of cDNA could not be detected in livers of tolerized animals that received transplanted cells but were not infected by virus (Fig. 8, lanes 7 and 8) or in livers of tolerized animals that did not receive the transplant but were infected with HBV (Fig. 8, lane 6).

**Conclusion**

Rats that were fetally tolerized to human hepatocytes could host human hepatocytes in the liver and were susceptible to HBV infection. Tolerized animals with chimeric human livers could also be used to develop a rat model for HCV.

**Acknowledgments**

This work was partly supported by grants from Connecticut Innovations, Inc. (CHW), the Herman Lopata Chair in Hepatitis Research (GYW), and NIDDK: DK-42182 (GYW).


**References**


Received: May 31, 2001
Accepted: June 28, 2001

Correspondence to:
Catherine H. Wu
Department of Medicine
Division of Gastroenterology-Hepatology
University of Connecticut Health Center
Rm. AN-045
263 Farmington Avenue
Farmington, CT 06030-1845, USA
cwu@nsol.uchc.edu