

A Novel cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} Mouse Model for Hepatitis Virus Infection and Reconstruction of Human Immune System

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Abstract

The humanized mouse is a widely used *in vivo* model for the investigation of pathogenesis and drug development. In this study, we generated new immunodeficiency cDNA-urokinase-type plasminogen activator (uPA)/SCID/Rag2^{-/-}/Jak3^{-/-} mice and established the mouse model with a humanized liver and immune system. Transplantation of human hepatocytes with human leukocyte antigen (HLA)-A24 resulted in establishment of a highly replaced liver in this mouse model. These mice were successfully infected with hepatitis B virus and hepatitis C virus (HCV) for a prolonged period and are available for the analysis of the effect of anti-HCV drugs. Administration of peripheral blood mononuclear cells (PBMCs) obtained from a donor with HLA-A24 resulted in the establishment of 22.6–81.3% of human CD45-positive mononuclear cell chimerism in liver infiltrating cells without causing graft versus host disease. When mice were transplanted with human hepatocytes and then administered PBMCs, an alloimmune response between transplanted human hepatocytes and PBMCs occurred, with production of transplanted hepatocyte-specific anti-HLA antibody. The alloimmunity was never inhibited by methylprednisolone nor cyclosporine A. In conclusion, we succeeded in establishing a humanized liver and immune system using a novel cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mouse. This model is not only useful to study hepatitis virus virology but also to study alloimmunity.

Introduction

The humanized mouse model is widely used for the exploration of disease pathogenesis and the evaluation of the therapeutic effect of drugs. Several immunodeficient mice have been developed in order to accept transplanted human cells. NOD/SCID/IL2γc^{-/-}, such as NOG and NSG mice, are considered a suitable platform for *in vivo* reconstruction of the human immune system. However, it is known that the genetic background of these mice is influenced by the efficiency of human cell engraftment¹. Rag2/Jak3 double-deficient (Rag2^{-/-}/Jak3^{-/-}) mice lacking mature T and B lymphocytes and NK cells, is a suitable mouse model for reconstruction of human immune system by transplantation of human immune cells².

Hepatitis B virus (HBV) and hepatitis C virus (HCV) pose a worldwide health burden causing acute hepatitis, chronic hepatitis, liver cirrhosis, and potentially death due to liver failure and hepatocellular carcinoma. The species tropism of these viruses is restricted to chimpanzee and human; thus, investigation of their pathology had been hampered by the lack of small animal model. Recently developed human hepatocyte replaced immunodeficiency mice, such as urokinase-type plasminogen activator (uPA)^{2,3}, herpes simplex virus type-1 thymidine kinase (HSVtk)⁴ transgene, or a targeted disruption of the murine fumaryl acetoacetate hydrolase (FAH)^{5,6} support hepatitis viruses infection and replication and are commonly used for the study of viral hepatitis^{7–9}. We previously reported that TK-NOG and cDNA-uPA/SCID mice were useful animal models for the study of HBV and HCV virology and the analysis of the effect of antiviral drugs^{10,11}. Moreover, we established the hepatitis B mouse model by the adoptive transfer of human immune cells to human hepatocyte replaced mice^{12,13}.

In this study, we generated novel cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice with humanized livers that were almost completely repopulated with human hepatocytes, and we are able to successfully infect these mice with HBV and HCV. In addition, we attempted to reconstruct elements of the human immune system in this novel mouse model by administration of human peripheral blood mononuclear cells (PBMCs).

Results

Characteristics of cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice

The pregnant ratio of cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice was very low when the Jak3 knockout was homozygous. Therefore, cDNA-uPA^{+/+,+/wt}/Rag2^{-/-}/Jak3^{+/-,-/-} mice were used for mating to obtain cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice. The male : female sex ratios of cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice were 4.1 : 5.9 and 3.5 : 6.5 for cDNA-uPA homozygous (n = 99) and hemizygous (n = 120), respectively. These ratios were similar to those for cDNA-uPA/SCID mice (4 : 6).

Repopulation of cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice with humanized livers

When the mice were 2–4 weeks of age, 5.0×10^5 viable human hepatocytes were isolated from human hepatocyte-replaced cDNA-uPA/SCID mice (PXB-mice[®]) by collagenase perfusion method and transplanted into cDNA-uPA^{+/+,+/wt}/Rag2^{-/-}/Jak3^{-/-} mice. A total of 89 cDNA-uPA^{+/+,+/wt}/Rag2^{-/-}/Jak3^{-/-} mice were transplanted with human hepatocytes. Changes in the body weight and serum HSA levels of 13-week-old mice are shown in Figure 1 and Table 1. The HSA levels increased steadily after transplantation of human hepatocytes and reached a steady state at around 11 weeks (Fig. 1A). Mice serum HSA levels were higher in cDNA-uPA homozygote chimeric mice compared to hemizygote chimeric mice from 6 weeks old (3 weeks after transplantation) to 13 weeks old, indicating that engraftment of human hepatocytes was superior in cDNA-uPA homozygote host mice. While body weights were higher in cDNA-uPA hemizygote mice than homozygote mice (Fig. 1B). Mean HSA levels in males were slightly higher than those in females of both cDNA-uPA homozygote and hemizygote human hepatocyte-replaced mice (Table 1). The correlation between HSA levels at 17 weeks old and the human hepatocyte replacement index, estimated by immunohistochemistry in cDNA-uPA homozygote mice, is shown in Figure 1B. HSA levels were exponentially correlated with replacement index in cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice ($y = 35.0 \ln(x) - 475.0$, $r^2 = 0.92$) in a manner comparable to PXB-mice[®] ($y = 29.9 \ln(x) - 397.0$, $r^2 = 0.88$)². The formula indicated that a level of more than 6.2 mg/mL HSA represents a replacement index exceeding 70% in human hepatocyte transplanted cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice.

Hepatitis virus infection to human hepatocyte-transplanted cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice

Each of six mice was inoculated with 1 μ L of serum samples containing 10^6 copies of HBV. Mice serum HBV DNA became positive two weeks after HBV inoculation, increased gradually, and reached 10^8 – 10^{10} copies/mL at 6–12 weeks (Fig. 2A). High levels of HBV DNA and HSA persisted until 30–37 weeks.

Each of four human hepatocyte-replaced cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice was injected with 10 µL of serum samples containing 10⁵ copies of HCV in order to establish HCV viremia. Mice serum HCV RNA became measurable two weeks after HCV inoculation and reached 10⁶–10⁸ copies/mL 10 weeks after HCV inoculation (Fig. 2B). When these mice were administered either the NS5A inhibitor daclatasvir or the NS3/4A protease inhibitor asunaprevir, viral breakthrough developed following a transient reduction in HCV RNA levels. These results indicate that HCV-infected cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice are useful for evaluating antiviral agents *in vivo* in a similar way to previous human hepatocyte chimeric mouse models.

Reconstruction of the human immune system in cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice

Human hepatocyte-transplanted cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice tolerate infection with hepatitis viruses and seem to be suitable animal models for evaluating the effect of antiviral drugs. However, the models are less likely to develop viral hepatitis due to the lack of an immune system. Human PBMCs were transplanted into cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice in order to establish a basic immune system. Characteristics of human PBMCs used in this study are shown in Table 2. First, 5 × 10⁶ PBMCs obtained from a healthy volunteer (Case 1) were administered into three cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice without human hepatocyte transplantation. Mouse body weight was maintained at baseline level after PBMC administration (Fig. 3A). Flow cytometry analysis revealed the establishment of 81.3% and 22.6% of human CD45-positive mononuclear cell chimerism in liver infiltrating cells 4 and 8 weeks after PBMC transplantation in two mice, respectively (Fig. 3B).

We next administered human PBMCs obtained from Case 1 (healthy volunteer) into four human hepatic hepatocyte-transplanted cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice. Mice developed rapid and severe weight loss, ruffled hair and reduced activity following PBMC transplantation (Fig. 4A). Flow cytometry analysis revealed an engraftment of 30.4% of human CD45-positive mononuclear cell in liver infiltrating cells in a mouse (Fig. 4B), and histological examination showed lymphocyte accumulation in livers, especially in human albumin-positive hepatocytes 19 weeks after PBMC transplantation (Fig. 4C). On the other hand, histological examination showed no evidence of development of graft versus host disease (GVHD) in multiple organs such as colon, small bowel, stomach, lung, kidney, pancreas, spleen and heart (Fig. 4D). Therefore, the symptoms following human PBMC administration seemed to be caused by alloimmunity between administered human PBMCs and transplanted human hepatocytes.

We also tested PBMC administration into HBV-infected cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice. Human hepatocyte-transplanted mice were infected with HBV and administered with PBMCs obtained from Case 1. Unfortunately, mice developed body weight loss, and mice serum HSA level decreased two weeks after PBMC administration similar to the experiment using uninfected mice. Although HBV DNA levels decreased in these mice in response to the reduction in HSA level, HBV-specific cytotoxic T lymphocytes (CTLs) were not detected in liver-infiltrating cells obtained from these mice (Fig. 5). These results indicate

that reduction of human hepatocytes in HBV-infected cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice was mainly due to the alloimmunity between administered human PBMCs and transplanted human hepatocytes.

To search for a candidate whose PBMCs do not induce an alloimmune response with transplanted human hepatocytes, we tested PBMCs obtained from a patient who had recovered from an episode of acute severe hepatitis B (Case 2) and a patient with chronic HBV infection (Cases 3) (Table 2). Similar to the PBMCs obtained from Case 1, PBMC administration resulted in reductions in body weight, HSA and HBV DNA level in both cases (Fig. 5).

To inhibit alloimmunity, mice were treated with either or both methylprednisolone and cyclosporine A for 7 days after PBMC transplantation. However, both mice body weight and HSA levels decreased in spite of methylprednisolone, cyclosporine A, or combination of these drugs treatments (Fig. 6).

We measured HLA antibodies in the serum of cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice after PBMC administration to make sure that an alloimmune response occurred between administered human PBMCs and transplanted human hepatocytes. Anti-HLA-B61 antibody, which matched the HLA type of the transplanted human hepatocytes, was detected in three mice that were administered serum from Donors 1 and 2 (Table 3). On the other hand, this antibody was not detected in any of the cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice that had not been transplanted with hepatocytes (data not shown). Although we detected non-specific anti-HLA antibodies in six out of ten mice that had been administered PBMCs, they were not detected in the remaining four mice. Non-specific anti-HLA antibodies detected in this study might be due to the presence of HLA-presenting cells in transplanted PBMCs. These results indicate that PBMC administration resulted in development of anti-HLA-B61 antibody and induced an alloimmune response to transplanted human hepatocytes in these mice.

Discussion

Human hepatocyte chimeric mice are widely used for research on HBV and HCV. Although this mouse model provides a platform for examining hepatitis virus infection, it is difficult to investigate the immune response to the viruses due to the lack of an immune system. In this study, we attempted to develop a novel chronic hepatitis B mouse model using human hepatocytes transplanted into severe immunodeficiency, cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice.

In human hepatocyte-transplanted cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice, characteristics of HSA levels and body weight in homozygotes and hemizygotes and in males and females were similar to those of PXB mice^{® 2}. HBV and HCV infection were supported in human hepatocyte-replaced cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice with sustained high HSA levels, and serum HBV DNA and HCV RNA reached similar levels to those in PXB-mice^{® 11}. Excellent repopulation of human hepatocytes resulted in high titers of viremia and allowed us to perform a long-term infection experiment in this mouse model. In addition, we administered daclatasvir and asunaprevir to HCV-infected human hepatocyte-replaced cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice and confirmed the antiviral effects. These results suggest that

this mouse model is useful for hepatitis virus infection experiments and the evaluation of antiviral agents in comparable way to PXB-mice®¹¹.

We next succeeded in engrafting human immune cells in cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} to reconstruct elements of a simple human immune system. Human immune cells were detected in mouse livers after PBMC administration, but mice developed the symptoms due to allo-response in human hepatocyte and immune cell dual chimeric mice with and without HBV infection. Although we tried to match the HLA haplotypes between human hepatocytes and PBMCs as much as possible, it was difficult to find candidates who have completely matched-HLA types. This phenomenon seemed to be mainly due to alloimmunity between transplanted human hepatocytes and administered human PBMCs because they were not observed in mice without human hepatocyte transplantation, and anti-HLA antibody was detected only in human hepatocyte transplanted mice. It is noteworthy that mismatch of HLA is one of the most important factors which led to the allo-reaction in this immune cell transplanted mouse model. Moreover, it is possible that an overreaction between human hepatocyte and transplanted PBMCs might be likely to occur due to the excellent repopulation of human hepatocytes.

For humanized hepatitis mouse models, it is reported that human hepatocyte and immune cells dual chimeric mouse model using hematopoietic stem cells obtained from cord blood could support HBV infection and investigation of human immune response to HBV-infected hepatocytes¹⁷. However, the human hepatocyte replacement ratio in this dual mouse model is considerably lower than in our chimeric mouse model. In addition, humanized mice with human induced pluripotent stem derived hepatic like cells are transplanted was reported¹⁸. Although stem cells also have the ability to differentiate into immune cells, the immune response model using stem cell derived immune cells is more complicated. Altogether, there are still many problems to overcome to establish a mouse model which mimic the viral hepatitis.

Meanwhile, the immune response between graft and host is a serious problem when constructing immune system *in vivo* models and is mainly thought to be caused by MHC class1/2 molecules¹⁹. Various xeno-immunity mouse models are reported using immunodeficiency mouse strains²⁰. However, no alloimmunity mouse model was reported so far. According to the previous report, allo-specific anti-HLA antibody was difficult to detect in human immune system chimeric mouse model without humanized liver²¹. In our mouse model, we confirmed immune cell accumulation, especially in human hepatocyte areas and detection of transplanted human hepatocyte-specific anti-HLA antibody. Antibody-mediated allograft rejection due to donor-specific anti-HLA antibody influences transplantation outcomes despite advances in immunosuppression and antibody screening prior to the transplantation^{22,23}. However, the mechanism of anti-HLA antibody-mediated rejection has not been fully elucidated so far. This mouse model might be helpful to investigate acute allo-reaction between transplanted human immune cells and human liver. Further study is needed to verify the mechanism of immune response in this mouse model.

In conclusion, we established a novel cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mouse model which supports reconstruction of hepatitis virus infection and part of the human immune system. This mouse model is useful for investigation of the pathogenesis of chronic hepatitis virus infection and evaluation of the effects of antiviral compounds with the potential to mimic donor-specific anti-HLA antibody-mediated alloimmunity.

Methods

Generation of cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice with humanized liver

Rag2^{-/-}/Jak3^{-/-} mice line with NOD, C57/BL6 and Balb/c genetic background were generated at Kumamoto University, Japan ¹. The generation of cDNA-uPA/SCID mice has been described previously ². cDNA-uPA/SCID mice were backcrossed twice with Rag2^{-/-}/Jak3^{-/-} mice with Balb/c genetic background. Subsequently, they were mated together by caesarian operation to obtain microbiologically cleaned cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice. To prepare host mice for human hepatocyte replaced mice, cDNA-uPA^{+/+ or +/wild} /SCID/ Rag2^{-/-}/Jak^{-/- or -/wild} mice were mated with each other. The genotypes of uPA, Rag2 and Jak3 were analyzed by the genomic PCR method. cDNA-uPA^{+/+ or +/wild}/SCID/Rag2^{-/-}/Jak3^{-/-} mice were transplanted with human hepatocytes with human leukocyte antigen (HLA)-A24 (BD Biosciences, Woburn, MA, USA) at 2–4 weeks old. The thawing and transplantation of human hepatocytes into host mice has been described previously in the generation of chimeric mice with human hepatocytes ⁴. All animal protocols described in this study were performed in accordance with the Guide for the Care and Use of Laboratory Animals (<https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf>) and the local committee for animal experiments, and the experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of the Graduate School of Biomedical Sciences, Hiroshima University and PhoenixBio Co., Ltd. This study is reported in accordance with ARRIVE guidelines (<https://arriveguidelines.org>).

Measurement of the replacement index and human serum albumin levels

Cryosections prepared from the liver (5 µm thick) were incubated with anti-human cytokeratin 8 and 18 (CK8/18) mouse monoclonal antibodies (*~100 dilution*, Cappel Laboratory, Cochranville, PA). Measurements of the replacement index of human hepatocytes to total human and mouse hepatocytes have been described previously ¹⁴. Mouse blood was collected from the tail vein. The concentration of human serum albumin (HSA) in mouse blood was measured using latex agglutination immunonephelometry (LX Reagent “Eiken” Alb II; Eiken Chseimical Co., Ltd., Tokyo, Japan).

HBV and HCV infections in mice

Twelve weeks after human hepatocyte transplantation, cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice were injected intravenously with either HBV or HCV containing serum. HBV containing serum was obtained from an HBeAg-positive patient with chronic genotype C HBV infection who had high virus titers (8.2 log

IU/mL). HCV containing serum was obtained from a patient with chronic genotype 1b HCV infection with high virus titers (6.3 log IU/mL). After serum inoculation, mouse blood samples were obtained serially, and serum viral titers and HSA levels were measured. Human serum samples were obtained from patients who provided written informed consent using a form that was made in accordance with the Declaration of Helsinki and was approved by the ethical committee of Hiroshima University. The individual serum samples were divided into small aliquots and stored separately in liquid nitrogen until use.

Quantification of HBV DNA and HCV RNA

Serum HBV DNA and HCV RNA levels were measured using the COBAS TaqMan test (Roche Diagnostics). The lower detection limits of the assay for HBV DNA and HCV RNA are 4.4 and 3.5 log copies/mL, respectively.

Treatment with direct-acting antivirals for HCV-infected mice

After establishment of stable viremia, HCV-infected cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice received orally either 40 mg/kg/day of asunaprevir (Bristol-Myers Squibb, New York, NY) or 10 mg/kg/day of daclatasvir (Bristol-Myers Squibb). The dose of each drug was determined as the mouse equivalent dose used in our previous report (15?).

Preparation of human PBMCs and transplantation into mice

PBMCs were isolated using Ficoll-Paque density gradient centrifugation according to the manufacturer instructions. 5×10^6 of PBMCs were transplanted into cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice by peritoneal injection.

Flow cytometry

We collected mouse liver-infiltrating cells flowing through the portal vein after hepatectomy¹⁶. Reconstructed human PBMCs in mice were analyzed by flow cytometry with monoclonal antibodies used for surface staining as described previously^{12,13}. Dead cells identified by light scatter and 7-AAD staining were excluded from the analysis. Flow cytometry was performed using a FACS Aria II flow cytometer (BD Bioscience, Franklin Lakes, NJ), and results were analyzed with FlowJo (BD Biosciences).

Histochemical analysis of mouse liver

Histochemical analysis and immunohistochemical staining using antibodies against human albumin (Bethyl Laboratories Inc., Montgomery, TX) were performed as described previously^{12,13}. Immunoreactive materials were visualized using a streptavidin-biotin staining kit (Histofine SABPO kit; Nichirei, Tokyo, Japan) and diaminobenzidine.

Treatment of mice with immunosuppression

One week after human PBMC transplantation, human hepatocyte-replaced cDNA-uPA/SCID/Rag2^{-/-}/Jak^{-/-} mice received either or both of 50 mg/kg/day of cyclosporine A orally and intra-peritoneal injections of 200 mg/kg of methylprednisolone for seven days.

Detection of anti-HLA antibody in mice

Anti-human leukocyte antigen (HLA) antibody was detected using WAKFlow classⅡ antibody specificity identification reagent (Wakunaga Pharmaceutical Co., Ltd., Tokyo, Japan) according to the manufacturer's protocol. Data acquisition and analysis were performed by the Luminex® system (Luminex corporation, Austin, TX) to identify HLA alleles encoded by sample DNA.

Abbreviations

DAAs, direct acting antivirals; FAH, fumaryl acetoacetate hydrolase; GVHD, graft-versus-host disease; HBV, Hepatitis B virus; HCV, Hepatitis C virus; HLA, human leukocyte antigen; HSVtk, herpes simplex virus type-1 thymidine kinase; PBMCs, peripheral blood mononuclear cells; uPA, urokinase-type plasminogen activator

Declarations

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Author contribution

Concept and design: TU, MI, and KC

Experiment, procedures and data analysis: TU, YT, MI, SH, YI and CT

Project administration: HF, AO, TN, EM, MY, TK, HAC, GNM, CNH, DM, AH, HA, YT, HO and KC

Provision of resources: SH, YI, CT and SO

Measurement of anti-HLA antibody: TS and SK

Writing of manuscript: TU, YT, MI, CNH, and KC

Supervision and critical revision of manuscript: CT, HO, SO and KC

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Conflict of interest statement

Michio Imamura has received research funding from AbbVie. Hiroshi Aikata has received honoraria from Eisai and Bayer. Kazuaki Chayama has received honoraria from Bristol-Myers Squibb and MSD K.K., AbbVie, Gilead Science, Dainippon Sumitomo Pharma and Mitsubishi Tanabe Pharma and research funding from Gilead Science, Dainippon Sumitomo Pharma, MSD K.K., AbbVie, Eisai, TORAY, Otsuka Pharma, Chugai Pharma, Takeda Pharma and Roche. Satoko Hamamura, Yuji Ishida and Chise tateno are employees of PhoenixBio Co., Ltd. Takayuki Shioruzu and Shintaro Kawai are employee of Wakunaga Pharmaceutical Co., Ltd.. All the other authors have no COI to declare.

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Tables

Table 1. Characteristics of cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice

uPA	Sex	n	Survival rate until 11 weeks (%)	Body weight at 11 weeks (g)	HSA level at 11 weeks ($\times 10^6$ ng/mL)	HSA level at 13 weeks ($\times 10^6$ ng/mL)
Homo	Male	8	100	15.7 \pm 2.3 (n=8)	9.6 \pm 1.0	10.7 \pm 1.3 (n=6)
	Female	17	88.2	14.6 \pm 2.4 (n=15)	8.7 \pm 1.5	10.1 \pm 1.4 (n=6)
Hemi	Male	23	100	17.4 \pm 3.8 (n=23)	6.8 \pm 2.1	8.2 \pm 1.8 (n=20)
	Female	41	97.6	16.9 \pm 2.5 (n=40)	5.8 \pm 2.0	7.4 \pm 1.5 (n=30)

uPA, urokinase-type plasminogen activator; HSA, human serum albumin

Table 2. Characteristics of peripheral blood mononuclear cells and hepatocytes

Case	Background	HBsAg	HBV DNA	HLA-A	HLA-B	HLA-DR
Case 1	Healthy volunteer	Negative	Undetectable	24:33	44:52	13:15
Case 2	Acute hepatitis B	Negative	Undetectable	24:24	52:52	15:15
Case 3	Chronic hepatitis B	Positive	Undetectable	24:24	52:54	4:15
BD195	Human hepatocyte	N.A.	N.A.	24:24	52:61	8:14

HBsAg, hepatitis B surface antigen; HLA, human leukocyte antigen; N.A., not applicable

Table 3. Anti-HLA antibody appearing after PBMC administration in human hepatocyte chimeric cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice.

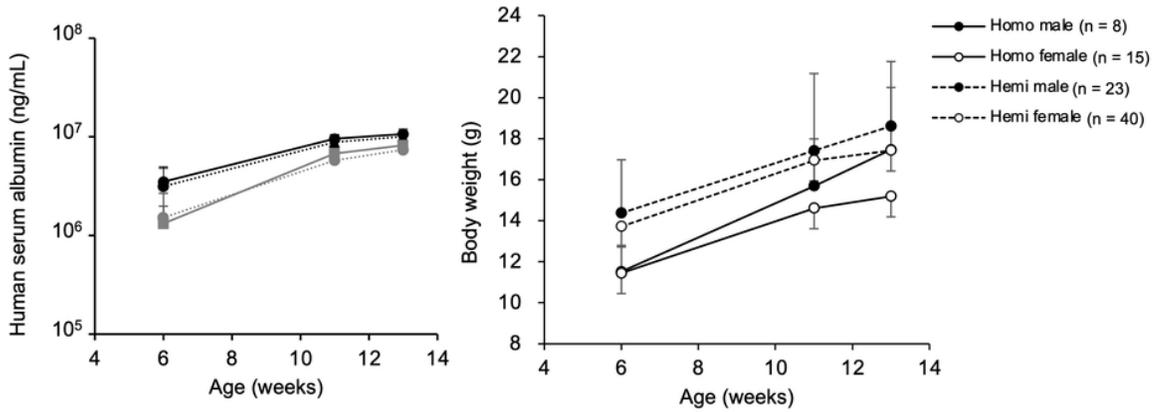
Donor	HBV infection	HLA-A	HLA-B
-	-	N.D.	N.D.
Donor 1	-	A11.1, A31, A30, A26	B61
Donor 1	-	N.D.	B54, B27, B62
Donor 1	+	N.D.	N.D.
Donor 1	+	N.D.	N.D.
Donor 2	+	N.D.	B7
Donor 2	+	N.D.	B61, B67
Donor 2	+	A31	B7, B61
Donor 2	+	N.D.	B37
Donor 3	+	N.D.	N.D.
Donor 3	+	N.D.	N.D.

B61, Anti-HLA antibody common7 to transplanted hepatocytes; N.D., not detected.

Figures

Figure 1

A



B

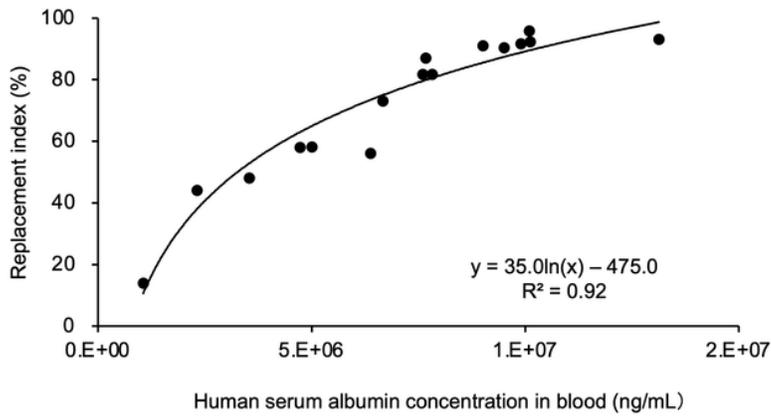


Figure 1

Human serum albumin concentrations and body weight in human hepatocyte-replaced cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice. cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice were transplanted with human hepatocytes. (A) Changes in human serum albumin concentrations and body weight in mice. (B) Correlation between replacement index and human serum albumin concentrations at 17-week-old.

Figure 2

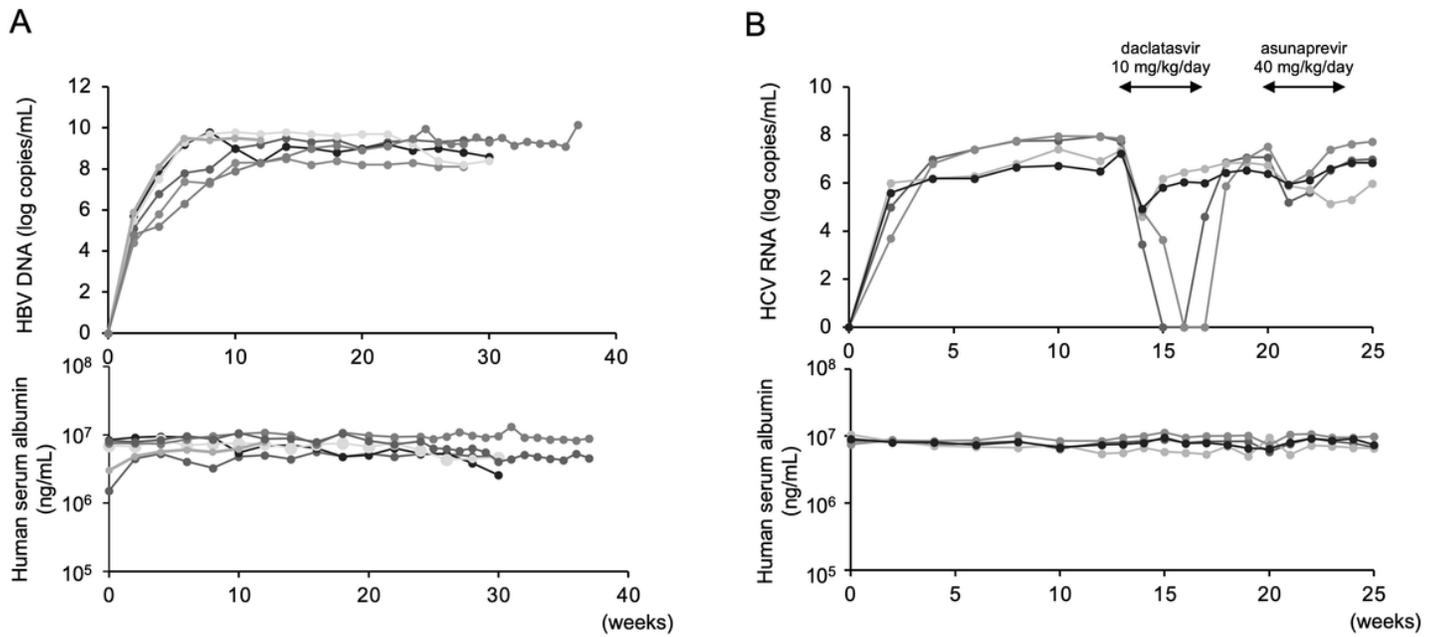


Figure 2

Hepatitis virus infection in human hepatocyte-replaced cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice. Ten human hepatocyte-transplanted cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice were infected with either HBV (A) or HCV (B). HCV-infected mice were treated with daclatasvir for four weeks and then treated with asunaprevir for four weeks. Changes in serum HBV DNA or HCV RNA titer and human serum albumin concentration in each mouse are shown.

Figure 3

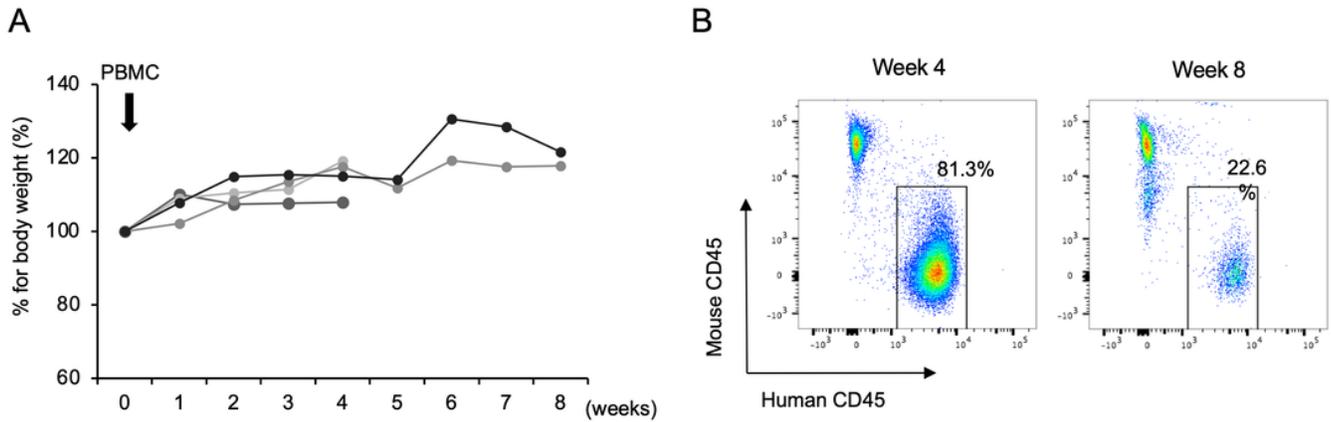


Figure 3

PBMC transplantation into cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice. Each of four cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice without transplanted human hepatocytes was administered human peripheral blood mononuclear cells (PBMCs) obtained from a healthy volunteer. (A) Changes in the percent of body weight in each mouse. (B) Proportions of human mononuclear cells in mouse liver infiltrating cells in two mice, at four and eight weeks after PBMCs transplantation, respectively.

Figure 4

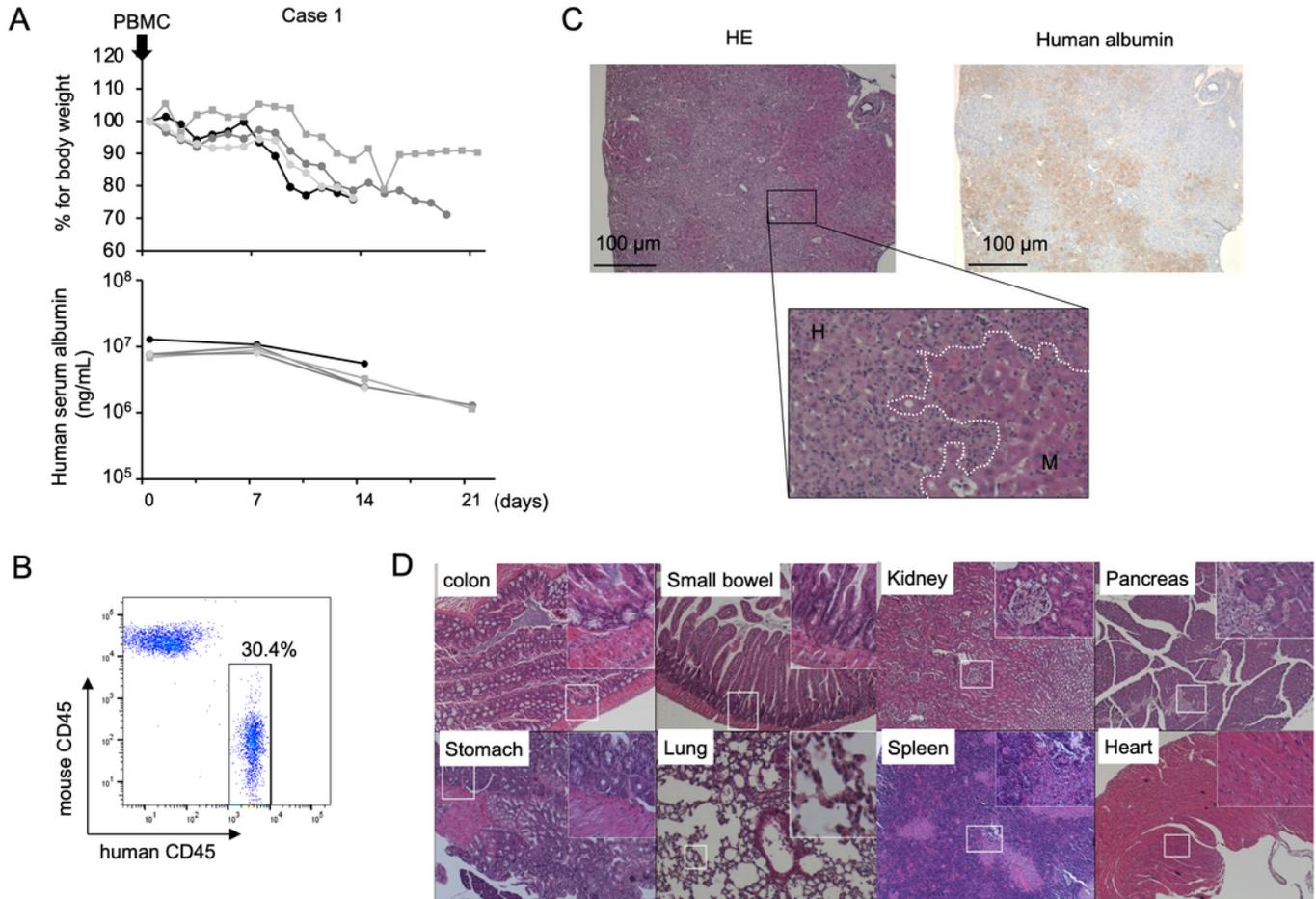


Figure 4

PBMC administration into human hepatocyte-transplanted cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice. Each of four human hepatocyte-transplanted cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice was administered human peripheral blood mononuclear cells (PBMCs) obtained from a healthy volunteer. (A) Changes in percent of body weight and human serum albumin concentration in each mouse. (B) Proportions of human mononuclear cells in mouse liver infiltrating cells. (C) Histological analysis of mouse liver samples. Liver samples at two weeks after injection of human PBMCs were stained with hematoxylin-eosin (HE) or anti-human albumin antibody. Regions are shown as human (H) and mouse (M) hepatocytes, respectively (original magnification, ×40 or ×200). (D) HE staining of colon, small intestine, kidney, pancreas, stomach, lung, spleen and heart (original magnification, ×40).

Figure 5

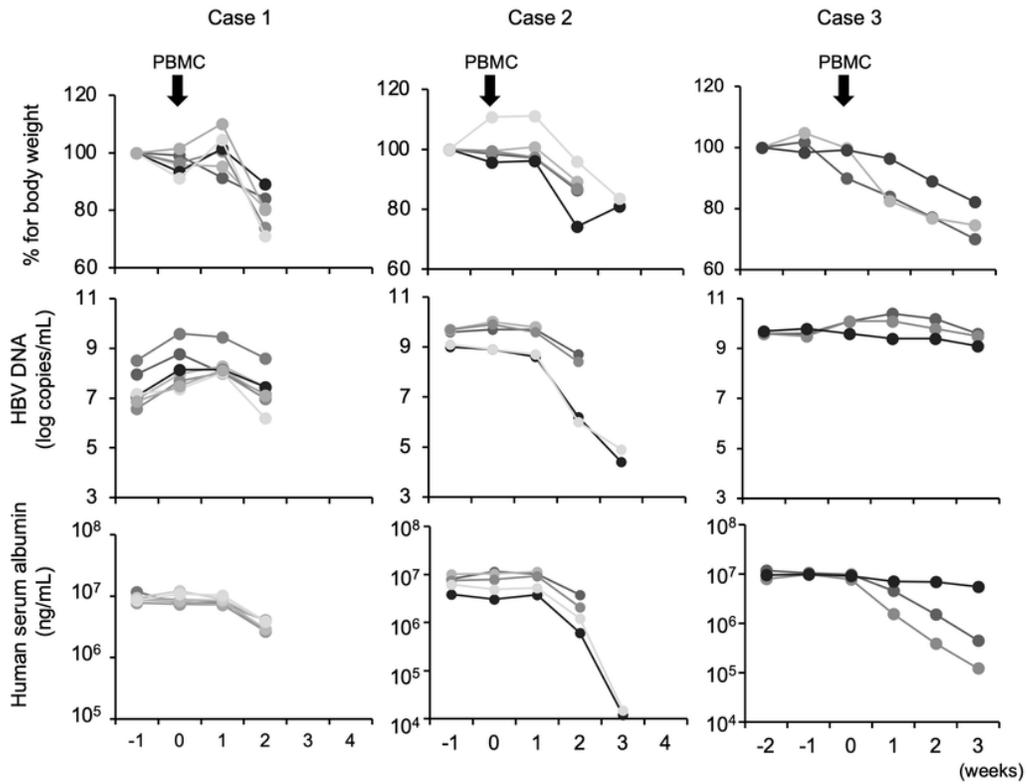


Figure 5

PBMC administration into HBV-infected cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice. Human hepatocyte-transplanted cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice were infected with HBV and administered human peripheral blood mononuclear cells (PBMCs) obtained from a healthy volunteer (Case 1), a patient recovered from an episode of acute hepatitis B (Case 2) or a chronic HBV-infected patient (Case 3). Changes in body weight, serum HBV DNA level and human serum albumin concentration in each mouse are shown.

Figure 6

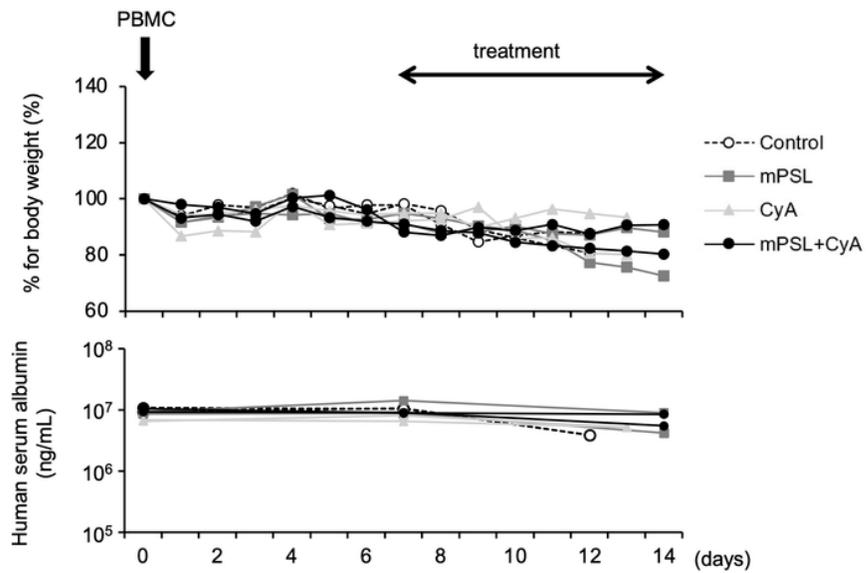


Figure 6

Treatment with immunosuppression for human PBMC-transplanted mice. Human hepatocyte-transplanted cDNA-uPA/SCID/Rag2^{-/-}/Jak3^{-/-} mice were administered human peripheral blood mononuclear cells (PBMCs) obtained from Case 1. One week after human PBMC transplantation, each of eight mice was treated with or without (Control) methylprednisolone (mPSL), cyclosporine A (CyA), or combination of mPSL and CyA for seven days. Changes in the percent of body weight and human serum albumin concentration in each mouse are shown.