Recurrence of Bile Salt Export Pump Deficiency after Liver Transplantation

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SUMMARY

Severe bile salt export pump (BSEP) deficiency is a hereditary cholestatic condition that starts in infancy and leads to end-stage liver disease. Three children who underwent orthotopic liver transplantation for severe BSEP deficiency had post-transplantation episodes of cholestatic dysfunction that mimicked the original disease. Remission of all episodes was achieved by intensifying the immunosuppressive regimen. The phenotypic recurrence of the disease correlated with the presence of circulating high-titer antibodies against BSEP that inhibit transport by BSEP in vitro. When administered to rats, these antibodies targeted the bile canaliculi and impaired bile acid secretion.

CHOLESTATIC DISORDERS ARE AMONG THE MOST SEVERE LIVER DISEASES in infancy and childhood. For some patients, orthotopic liver transplantation is the only effective therapy, resulting in favorable outcomes and no recurrence of the original disease. Severe BSEP deficiency, also referred to as progressive familial intrahepatic cholestasis type 2, is one such disorder. It is caused by recessive mutations in ABCB11, the gene encoding BSEP. BSEP is expressed at the canalicular membrane of the hepatocytes and transports bile acids into the canalicular space, using ATP as an energy source. Hepatocyte canaliculi in most patients carrying ABCB11 mutations express little or no detectable BSEP.

Children with severe BSEP deficiency typically have jaundice and pruritus within the first few months of life. Early-onset cholestasis progresses to hepatic fibrosis, cirrhosis, and end-stage liver disease. Affected children are also at increased risk for liver cancer. Biochemical and histopathological features of this disorder include elevated serum concentrations of bile acids, intrahepatic cholestasis, and often, giant-cell transformation. Serum values of γ-glutamyltransferase (GGT) activity are normal, despite the degree of conjugated hyperbilirubinemia. Liver disease caused by severe BSEP deficiency is usually resistant to medical treatment; therefore, for most patients, transplantation becomes necessary.

We report on a rare phenotypic recurrence of BSEP deficiency that takes place after liver replacement. The recurrence correlates with the presence in serum of blocking antibodies against BSEP.
METHODS

We obtained approval for the study from a local ethics committee. Written informed consent was provided by the patients or, if the patients were under 18 years of age, their parents.

SEQUENCE ANALYSIS OF ABCB11

We isolated genomic DNA from peripheral-blood leukocytes, using the PureGene DNA Isolation kit (Gentra Systems), and amplified all 28 exons of ABCB11 and flanking intron–exon boundaries by performing polymerase-chain-reaction assays. (Primer sequences and assay conditions are available on request.) We sequenced both strands using the 1.1 Big Dye Terminator RRMix (Applied Biosystems).

IMMUNOHISTOCHEMICAL ANALYSIS

Immunohistochemical studies were carried out on paraffin-embedded explanted liver samples and needle-biopsy specimens obtained during post-transplantation cholestatic episodes. Detection of BSEP and multidrug resistance–associated protein 2 (MRP2) was performed by means of the antigen-retrieval method, as previously described, with a mouse monoclonal anti-MRP2 antibody (clone M2III-6, Chemicon International) and a goat polyclonal anti-BSEP antibody (Santa Cruz Biotechnology) as primary antibodies.

INDIRECT IMMUNOFLUORESCENCE ANALYSIS

Serum samples from the patients were screened at dilutions ranging from 1:40 to 1:5120, with the use of commercially prepared sections from rat liver, kidney, and stomach (Euroimmun, Lübeck). Bound antibodies were detected with the use of fluorescein isothiocyanate (FITC)–conjugated rabbit antihuman IgA, IgG, and IgM antibodies (DakoCytomation).

### Table 1. Characteristics of the Three Patients with Recurrent Severe Bile Salt Export Pump Deficiency after Liver Transplantation.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>M</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>Age at transplantation (yr)</td>
<td>5.2</td>
<td>3.7</td>
<td>2.2</td>
</tr>
<tr>
<td>ABCB11 mutation</td>
<td>Homozygote, 907A→G</td>
<td>Compound heterozygote, 1741C→T and IVS12+1G→T</td>
<td>Homozygote, IVS17+1T→A</td>
</tr>
<tr>
<td>Predicted amino acid mutation</td>
<td>R303G</td>
<td>L581F and splice-site disruption</td>
<td>Splice-site disruption</td>
</tr>
</tbody>
</table>

**Figure 1 (facing page).** Characteristic Findings of Episodic Cholestasis after Liver Transplantation for Severe Bile Salt Export Pump (BSEP) Deficiency in Patient 2.

Panel A shows immunohistochemical detection of BSEP and multidrug resistance–associated protein 2 (MRP2) in a liver specimen from a child without cholestasis (control) and the explanted liver from Patient 2. Paraffin-embedded sections were stained with primary antibodies at a 1:20 dilution. Sections were counterstained with hematoxylin. BSEP staining was also absent in Patients 1 and 3. Panel B shows the results of liver-function testing during the course of the first post-transplantation cholestatic episode in Patient 2. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, and γ-glutamyl transpeptidase (GGT) levels were monitored from the time of onset of the cholestatic attack (day 0) to the time of recovery. GGT activity remained relatively constant despite the long-lasting period of jaundice. Panel C shows morphologic characteristics of the liver-biopsy specimen obtained from Patient 2 at 26 days after the onset of the first post-transplantation cholestatic attack. Multinucleated giant hepatocytes (arrows) are visible (hematoxylin and eosin). The inset shows a portal tract in the same specimen, with a moderately dense lymphocytic infiltrate but no evidence of damage to the bile duct or portal vessels. The left-hand image in Panel D shows the pattern of immunofluorescence staining of rat-liver sections incubated with serum (dilution, 1:640) obtained at the onset of the third episode of cholestatic dysfunction in Patient 2. No reaction was found in sections of rat kidney or stomach. Positive bile-canicular staining was also detected with the use of serum samples from Patients 1 and 3. The right-hand image in Panel D shows the result of indirect immunofluorescence analysis of serum from a control (Patient 4) obtained during the second episode of acute cellular rejection (see the Supplementary Appendix for details). Panel E shows serum antibody titers during the fifth cholestatic attack in Patient 2. The intensity of the immunosuppressive regimen was increased on day 0. Resolution of pruritus was achieved on day 15. On complete recovery of normal liver function, the anticanalicular antibody titer was 6.25% of that at the peak of the cholestatic attack. (Titers are indicated within the plot.) For Panels B and E, to convert values for bilirubin to micromoles per liter, multiply by 17.1.
**Western-Blot Analysis**

Membrane vesicles (containing a total of 30 μg of protein) from Sf9 cells (derived from *Spodoptera frugiperda*) expressing either human BSEP (Sigma-Aldrich) or human MRP1 (BD Biosciences) were fractionated by means of 10% sodium dodecyl sulfate–polyacrylamide-gel electrophoresis. The gels were transferred onto polyvinylidene difluoride membranes and cut into strips, which were probed with serum samples from patients (at a 1:100 dilution) or with the goat antibody against human BSEP (at a 1:200 dilution). Immune complexes were detected by means of horseradish peroxidase–conjugated goat antihuman immunoglobulins (1:16,000 dilution, Nordic Immunological Laboratories) or rabbit antigoat immunoglobulins (1:6000 dilution, DakoCytomation).

**Evaluation of Functional Transport**

Uptake of $^{3}$H-labeled taurocholate (American Radiolabeled Chemicals) in membrane vesicles from Sf9 cells expressing human BSEP was measured...
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Episode 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time after transplantation (yr)</td>
<td>12.0</td>
<td>3.5</td>
<td>5.2</td>
<td>8.1</td>
<td>12.1</td>
<td>13.0</td>
<td>2.1</td>
<td>4.0</td>
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<tr>
<td>Precipitating factors</td>
<td>Unknown</td>
<td>EBV infection</td>
<td>Unknown</td>
<td>Withdrawal of corticosteroids</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Low cyclosporine (trough level, &lt;50 ng/ml)</td>
<td>Switch from tacrolimus to cyclosporine for food allergy</td>
</tr>
<tr>
<td>Immunosuppressive therapy before episode</td>
<td>Cyclosporine (trough level, 63 ng/ml), azathioprine (1.1 mg/kg/day), methylprednisolone (0.1 mg/kg/day)</td>
<td>Methylprednisolone (0.43 mg/kg on alternate days), cyclosporine (trough level, 81 ng/ml)</td>
<td>Tacrolimus (trough level, 0.14 mg/kg on alternate days), azathioprine (0.9 mg/kg/day)</td>
<td>Tacrolimus (trough level, 0.15 mg/kg on alternate days), mycophenolate mofetil (1.2 mg/kg/day)</td>
<td>Tacrolimus (trough level, 0.09 mg/kg/day), mycophenolate mofetil (1100 mg/m^2/day)</td>
<td>Cyclosporine (trough level, 0.26 mg/kg on alternate days)</td>
<td>Cyclosporine (serum level 2 hr after injection, 678 ng/ml), methylprednisolone (0.26 mg/kg on alternate days)</td>
<td></td>
</tr>
<tr>
<td>Symptoms</td>
<td>Jaundice, pruritus</td>
<td>Jaundice, pruritus</td>
<td>Jaundice, pruritus</td>
<td>Jaundice, pruritus</td>
<td>Asthenia</td>
<td>Pruritus</td>
<td>Pruritus</td>
<td>Jaundice, pruritus</td>
</tr>
<tr>
<td>Markers of liver function†</td>
<td>Bilirubin (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>30.2</td>
<td>7.6</td>
<td>2.5</td>
<td>4.4</td>
<td>1.5</td>
<td>0.9</td>
<td>2.5</td>
<td>9.4</td>
</tr>
<tr>
<td>Direct (conjugated)</td>
<td>21.6</td>
<td>4.9</td>
<td>1.7</td>
<td>2.3</td>
<td>Not assessed</td>
<td>0.2</td>
<td>1.7</td>
<td>7.0</td>
</tr>
<tr>
<td>AST (U/liter)</td>
<td>320</td>
<td>190</td>
<td>42</td>
<td>220</td>
<td>515</td>
<td>150</td>
<td>110</td>
<td>280</td>
</tr>
<tr>
<td>ALT (U/liter)</td>
<td>810</td>
<td>220</td>
<td>30</td>
<td>205</td>
<td>1415</td>
<td>360</td>
<td>150</td>
<td>300</td>
</tr>
<tr>
<td>GGT (U/liter)</td>
<td>35</td>
<td>37</td>
<td>7</td>
<td>33</td>
<td>38</td>
<td>16</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>Histologic features</td>
<td>At day 21: cholestasis, multinucleated hepatocytes</td>
<td>At day 26: ductopenia, multinucleated hepatocytes</td>
<td>At onset: minimal cytolysis, normal ducts</td>
<td>At onset: multinucleated hepatocytes</td>
<td>At onset: multinucleated hepatocytes</td>
<td>Not assessed</td>
<td>At day 30: mild fibrosis, ductopenia</td>
<td>At onset: multinucleated hepatocytes</td>
</tr>
<tr>
<td>Endotheliitis or bile duct damage from inflammation</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Not assessed</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>
in the presence and in the absence of 4 mM ATP, as previously described, with the use of a rapid filtration assay. For inhibition studies, membrane vesicles were preincubated at 37°C for 30 minutes in the presence of serum samples from patients (dilution, 1:75) or 20 μM cyclosporine.

**IN VIVO STUDIES**

Four groups of three male rats (Sprague–Dawley rats, 4 weeks of age, weighing 60 to 65 g) were injected in the tail vein on two consecutive days with 0.2 ml of phosphate-buffered saline alone, phosphate-buffered saline containing 40 μg of a rabbit polyclonal antibody against human BSEP (H-180, Santa Cruz Biotechnology), 10 mg of human IgG (Grifols), or filter-sterilized serum from Patient 2 at an antibody titer of 1:5210. Twenty-four hours after the last injection, we anesthetized the animals with isoflurane, collected specimens of their bile through a cannula placed in the common bile duct for 15 minutes, and then performed exsanguination through the ascending cava. We assayed total bile acids using the 3α-hydroxysteroid dehydrogenase method and tested for differences among the four rat groups using Student’s t-test. Liver-tissue sections (thickness, 4 μm) were incubated for 1 hour with secondary antibodies (AlexaFluor488-conjugated antirabbit IgG at a 1:100 dilution, or FITC-conjugated antihuman IgA, IgG, and IgM antibodies at a 1:20 dilution). The same sections were subsequently incubated with a mouse anti-MRP2 antibody (1:100 dilution) and antimouse AlexaFluor594-conjugated secondary antibodies (Molecular Probes). These procedures were approved by our institutional animal care and use committee.

**Results**

Three children who had clinically diagnosed progressive familial intrahepatic cholestasis underwent liver transplantation involving a deceased donor. Several years later, they had episodes of graft dysfunction that mimicked the original disease. Mutational analysis of ABCB11, carried out in each of the children after transplantation (Table 1), and immunohistochemical studies of paraffin-embedded archival hepatectomy specimens (Fig. 1A) indicated that severe BSEP deficiency, a form of progressive familial intrahepatic cholestasis in which GGT activity is low, was the cause of the disease.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Disappearance of jaundice</th>
<th>Outcome of liver function testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Switch to tacrolimus (trough level, 7 ng/ml), corticosteroids (10 mg/kg/day)</td>
<td>Normal at 4 mo</td>
<td>Normal at 3 mo</td>
</tr>
<tr>
<td>Switch to tacrolimus (trough level, 10 μg/ml), high-dose corticosteroids (10 mg/kg/day)</td>
<td>Normal at 2 mo</td>
<td>Normal at 1 mo</td>
</tr>
<tr>
<td>Switch to tacrolimus (trough level, 15 μg/ml), high-dose corticosteroids (10 mg/kg/day)</td>
<td>Normal at 4.5 mo</td>
<td>Normal at 4 mo</td>
</tr>
<tr>
<td>Controversial corticosteroids (10 mg/kg/day)</td>
<td>Normal at 3 mo</td>
<td>Normal at 3 mo</td>
</tr>
<tr>
<td>Controversial corticosteroids (10 mg/kg/day)</td>
<td>Normal at 2 mo</td>
<td>Normal at 1 mo</td>
</tr>
</tbody>
</table>

* The assessment of antibodies and in vitro studies of bile acid transport were carried out with the use of serum samples obtained at the onset of cholestasis. EBV denotes Epstein-Barr virus. Normal values are as follows: total bilirubin, less than 1.1 mg per deciliter (19 μmol per liter); direct bilirubin, less than 0.3 mg per deciliter (51 μmol per liter); ascpartate aminotransferase (AST), less than 50 U per liter; alanine aminotransferase (ALT), less than 45 U per liter; and γ-glutamyl transpeptidase (GGT), less than 30 U per liter. To convert values for bilirubin to micromoles per liter, multiply by 17.1.
Episodes of pruritus or jaundice with pruritus were the first symptoms to occur after transplantation in each of the children. (See Table 2 for other clinical, biochemical, and histologic features of the patients’ disease.) The clinical course of each episode was characterized by elevated serum values of aminotransferases and bilirubin but normal GGT activity (Fig. 1B). Graft-biopsy specimens obtained during periods of such elevations did not show signs of acute rejection (e.g., inflammatory bile-duct damage or endothelialitis). Some of the biopsy specimens showed intrahepatic cholestasis and giant-cell transformation (Table 2 and Fig. 1C); on immunohistochemical analysis, the specimens had normal levels of BSEP expression. The onset of cholestasis was associated in some instances with a reduction of immunosuppressive therapy in response to infection with the Epstein–Barr virus or food allergy. We could not ascertain the precipitating factor in four episodes of cholestasis. In all cases, an increase in immunosuppression, as detailed in Table 2, led to the resolution of symptoms.

Serum samples obtained from the three children during the cholestatic attacks were tested for the presence of typical autoantibodies. None of the samples were found to be positive. However, all of them contained high titers of antibodies (range, 1:1280 to 1:5120) that specifically reacted with antigens in bile canaliculi, as assessed by means of indirect immunofluorescence (Fig. 1D). Serum specimens were available from Patients 2 and 3 when they were asymptomatic; in these samples, the antibody titer fluctuated between 1:40 and 1:160. The antibody titer was also monitored during the course of the fifth post-transplantation cholestatic episode in Patient 2. The titer gradually declined from 1:5120 to 1:320 concomitantly with the normalization of the patient’s clinical condition and liver chemical values (Fig. 1E).

To test the hypothesis that these canalicular antibodies were directed against BSEP, serum samples from each of the three patients were analyzed by means of immunoblotting with membrane proteins from insect SF9 cells expressing either human BSEP or human MRP1. A polyclonal antibody against human BSEP immunoreacted with a protein of approximately 140 kD in membrane lysates from BSEP-expressing SF9 cells. All serum samples predominantly immunoreacted with a protein of the same molecular mass (Fig. 2A). This immunoreactive band was not visualized in control lysates from MRP1-expressing cells.

To substantiate an association between cholestatic graft dysfunction and the presence of anti-BSEP antibodies, we tested the serum samples from a control group of children (Patients 4, 5, and 6) who had undergone liver transplantation for severe BSEP deficiency and who did not have recurrence of the disease over a mean follow-up period of 5.5 years (see the Supplementary Appendix, available with the full text of this article at NEJM.org). Serum samples from two children who had undergone liver transplantation for extrahepatic biliary atresia and had been healthy for 5 years after transplantation were used as additional controls. None of the control samples were positive for anticanalicular antibodies (Fig. 1D) or anti-BSEP antibodies (Fig. 2A).

We then determined whether circulating anti-BSEP antibodies could interfere with BSEP function in grafts, by using an assay of bile acid transport with inside–out membrane vesicles from BSEP-expressing SF9 cells. The ATP-dependent vesicle uptake of $^3$H-labeled taurocholate (mean ±SD, 27.5±1.7 pmol per milligram of protein per minute) was not affected by preincubation with serum samples from any of the controls but was completely inhibited by preincubation with cyclosporine, a competitive inhibitor of BSEP, and by preincubation with serum samples from each of Patients 1, 2, and 3 (Fig. 2B).

We next tested the ability of these antibodies to reach the bile canaliculus and impair bile acid secretion in vivo. We intravenously injected rats with phosphate-buffered saline or with a rabbit antibody raised against human BSEP that cross-reacts with rat Bsep. The rats were killed, and sections of their livers stained with fluorescent antirabbit secondary immunoglobulins. We found no specific staining of liver sections from rats injected with phosphate-buffered saline, whereas those from rats injected with the antibody against BSEP showed canalicular staining (Fig. 3A). Sequential incubation of the same liver sections with an antibody against the canalicular protein MRP2 further confirmed that the injected anti-BSEP antibody localized predominantly on the canalaricular domains. Similarly, after injecting rats with serum from Patient 2 and then incubating liver sections with fluorescein-conjugated antihuman secondary antibodies, we observed staining of bile canaliculi, whereas there was no staining in sections from control animals injected with human IgG (Fig. 3A). Rats injected with anti-BSEP antibody or with serum from Patient 2 had biliary concentrations of...
total bile acids that were 50.0 to 62.5% of the levels in the corresponding controls. Bile acid concentrations in serum from these animals were significantly greater than the values in serum from control rats (Fig. 3B).

**DISCUSSION**

We describe late, post-transplantation phenotypic recurrence of severe BSEP deficiency in three children with an immune response against BSEP. The fact that anti-BSEP antibodies in one of these children bound the canalicular membrane of hepatocytes when injected into rats was not unexpected; it has been shown that IgG can be internalized by hepatocytes and even transcytosed from blood to bile. Moreover, in the two children tested, the antibody titer in the interval between episodes was markedly lower than that during the cholestatic bouts. The affected children were not tested for anti-BSEP antibodies before liver transplantation; all of them received transplants before ABCB11 was iden-

![Figure 2. Reactivity of Serum from the Patients to Human Bile Salt Export Pump (BSEP) and Blockade of Bile Acid Transport Function In Vitro.](image)

Panels A and B show the results of analyses of serum samples from children who had undergone liver transplantation for severe BSEP deficiency and who had disease recurrence after transplantation (Patients 1, 2, and 3) and from children without recurrence after transplantation (controls [Patients 4, 5, and 6]), as well as pooled serum samples from two children with extrahepatic biliary atresia (EHBA). Membrane vesicles (containing a total of 30 μg of protein) from Sf9 cells expressing either human BSEP or human multidrug resistance–associated protein 1 (MRP1), as a control, were fractionated through 10% sodium dodecyl sulfate–polyacrylamide-gel electrophoresis and subjected to Western-blot analysis (Panel A) with a goat antibody against human BSEP (dilution, 1:200) or with serum samples (dilution, 1:100) from patients and controls. Visualization was performed with the use of horseradish peroxidase–conjugated goat antihuman IgG or rabbit antigoat IgG. The arrowhead indicates the immunoreactive band obtained with use of the anti-BSEP antibody in membrane lysates from BSEP-expressing Sf9 cells. Panel B shows the results of analysis of ATP-dependent 3H-labeled taurocholate uptake in membrane vesicles containing human BSEP in the presence of phosphate-buffered saline (PBS), cyclosporine (20 μM), or serum samples from patients and controls (dilution, 1:75). The uptake of 3H-labeled taurocholate was measured by incubating samples for 10 minutes at 37°C in the presence and absence of 4 mM ATP. Each data point represents the mean value of triplicate measurements. On average, the levels of cyclosporine in diluted serum samples were 1/20,000 of the levels in the control sample used to test for drug-induced inhibition of BSEP activity. T bars and I bars indicate standard deviations.

Our results support the notion that phenotypic recurrence of severe BSEP deficiency results from an antibody-mediated blockade of BSEP in grafts. This is consistent with findings derived from the sequential assessment of antibody titers during the course of one episode of cholestatic graft dysfunction, in which normalization of clinical conditions and the results of liver tests under an increasingly intense immunosuppressive regimen were accompanied by a gradual decrease in the antibody titer. Moreover, in contrast with humans, rodents have canalicular bile acid transporters other than Bsep.18,19

We did not find a uniform distribution of staining by these antibodies among tissue sections. (Moreover, in contrast with humans, rodents have canalicular bile acid transporters other than Bsep.18,19)
tified as the gene causing this disorder. Anti-BSEP antibodies are unlikely to account for the disease in the native liver. In the three patients, the original disease can be accurately ascribed to a direct defect in BSEP, as determined by sequence analysis and immunohistochemical studies. The antibodies probably developed after liver transplantation, in response to foreign BSEP acquired with the graft. Epitopes present on wild-type BSEP introduced with the graft seem likely to be recognized as new antigenic determinants by the immune system of the recipient. None of the mutations present in Patients 1, 2, and 3 have been found in other children with severe BSEP deficiency.5

There are a few reports of a humoral response, after solid-organ transplantation, against the disease-causing gene product. Circulating antibodies

Figure 3. Deposition of Anti–Bile Salt Export Pump (BSEP) Antibodies or Serum from Patients on the Bile Canaliculi and Impairment of Biliary Secretion of Bile Acids.

Rats were injected intravenously on each of 2 consecutive days with a rabbit anti-human BSEP antibody (three rats) or with serum from Patient 2 (three rats). Phosphate-buffered saline (PBS) and human IgG were administered as respective controls (three rats per group). Bile, serum, and liver samples were collected 24 hours after the last injection. Panel A shows liver-tissue sections that were first stained with anti-rabbit or anti-human secondary antibodies (green) and were then incubated with a mouse antibody against multidrug resistance–associated protein 2 (MRP2) followed by antimouse AlexaFluor594–conjugated secondary antibodies (red). Liver specimens from antibody-treated rats show immunofluorescence-reaction products colocalized with the canalicular marker MRP2. Representative immunofluorescence images of liver sections from each group are also shown. Panel B shows the mean total bile acid concentrations in bile and serum specimens from the rats. T bars indicate standard deviations.
against dystrophin were detected in a patient with Becker’s muscular dystrophy (caused by mutations of the dystrophin gene); the patient underwent cardiac transplantation and then had graft failure. In a minority of patients who undergo kidney transplantation for Alport’s syndrome, a hereditary disease associated with mutations in one or several subunits of type IV collagen of the basement membranes, the presence of post-transplantation antibodies against the normal type IV collagen of the graft correlates with the development of glomerulonephritis.

Our current findings constitute a rare phenomenon among liver diseases for which transplantation is indicated in the pediatric population, because such diseases rarely recur after transplantation. We recommend that children who are undergoing liver transplantation for BSEP deficiency be checked for circulating anti-BSEP antibodies. If the patients are positive for the antibodies, special attention must be given to the management of immunosuppressive therapy.

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