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Serological Studies of Adenoviral Hepatitis Following Pediatric Liver Transplantation

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INFECTIONS with the herpes group of viruses are the most common viral infections following pediatric liver transplantation.[1] Adenoviral infections, though uncommon, are being increasingly recognized following organ transplantation.[2–5] We have previously reported our initial experience with adenoviral infections in children receiving liver transplants.[6] Subsequently, our experience has further increased and is being reported to amplify our initial observations. Also, serological studies of donor and recipient sera were performed to help understand the type and mode of infection. An attempt was made to correlate these data to patients' clinical course.

MATERIALS AND METHODS

During a 7-month period. 10 of 393 (2.5%) pediatric liver recipients developed adenoviral hepatitis. Diagnosis was made by percutaneous liver biopsy in 9 patients (90%). Histological features were characteristic with circumscribed foci of necrosis in the hepatic lobule with infiltration of monocytes. Diagnosis was confirmed by immunohistochemical staining.

Donor sera were obtained from blood samples drawn at the time of organ procurement. Recipient sera were obtained from blood samples drawn just prior to the transplant. Sera were stored frozen at −20°C. Portions of each serum available for viral antibody study were coded, refrozen (−70°C), and later thawed for anonymous, simultaneous testing.

Neutralization Tests

Adenovirus type specific neutralizing antibody titers were determined against known adenovirus types I through 5 for all samples in the study. Briefly, serial 2-fold dilutions of each sample were made in duplicate in 96-well plastic tissue culture plates (Nunclon, Gibco) in 0.025 mL volumes, using complete cell culture maintenance medium. Each well received 0.025 mL of the respective adenovirus type, diluted to contain from 300 to 1000 TCID50 units of virus in 0.025 mL. Plates were placed in a humidified chamber of a standard cell culture incubator for up to 7 days. Results were read at the time of appearance of specific cytopathic effect (CPE). Assignment of neutralizing titer was made using the highest of the paired serum dilutions (wells) showing absence of CPE.

Adenoviruses

Each of the adenovirus types were purchased from the American Type Culture Collection (Bethesda, MD), and verified as to serotype against 2 to 8 neutralizing units of a known type-

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specific serum (CDC source: Dr W. McD. Hammon). The adenovirus strains used were: type-1 VR-1, type-2 Adenoid 6, type-3 GB, type-4 RI-67, and type-5 Adenoid 75.

Cells and Media

HEP-2 (human laryngeal epidermoid carcinoma cell line) were grown in plastic flasks to confluent monolayers. Cell growth medium was Eagle's Minimum Essential Medium supplemented with 8% inactivated newborn calf serum, 25 mmol/L Hepes, 100 units of penicillin G and 100 μg of streptomycin sulfate per mL. Cells were verified to be free of mycoplasma contamination, by Hoechst 33258 staining at the time of use.

RESULTS

Neutralizing antibody titers of 1:4 or greater were considered positive. Antibody titers to only the relevant serotype in a given patient are shown in Table 1.

Of the 10 patients with the A V hepatitis, 3 recovered spontaneously with lowering of immunosuppression (patients 2, 3, and 10). Two of these 3 patients had high titers of type specific neutralizing antibodies at transplantation. Four progressed to massive liver necrosis (patients 4, 5, 6, and 9) and only 1 among them had neutralizing antibodies at a low titer of 1:4. Retransplantation was attempted in 3 of the patients with massive liver necrosis, 2 succumbing during surgery. Interestingly, the lone survivor did not develop recurrent A V hepatitis. The remaining 3 (patients 1, 7, and 8) had coexisting problems (lymphoproliferative disorder, chronic rejection, and abdominal sepsis). Only 1 of these 3 patients survived following retransplantation for combined rejection and A V hepatitis. This patient also did not develop recurrence of hepatitis.

DISCUSSION

Adenoviral infections are being increasingly recognized in organ transplant recipients.[2–5] In our previous report we described the features of 5 cases of A V hepatitis among a total of 22 children with A V infections after liver transplantation.[6] Further experience since then has corroborated our initial clinico-pathological observations. Even though A V hepatitis occurred in only 2.5% of all pediatric liver recipients it was the second most common viral infection of the hepatic allograft next only to cytomegalovirus infections.

Histological features are characteristic and immunohistochemistry confirms the diagnosis very quickly. With increasing experience we have relied less on electron microscopic demonstration of the virus. Culture of the virus is not necessary for the diagnosis, but it should be pursued for serotyping and other studies. Although several serotypes have been shown to cause hepatitis, serotype 5 has been the most common pathogen in the bone marrow recipients and also in our experience.

All cases of A V hepatitis with available serological data in our series show that either the donor or the recipient were seropositive at the time of the transplant. This supports our belief that the infection is either transmitted through the donor organ or is a reactivation of a latent recipient infection. The majority of our recipients (67%) were seronegative at the time of the transplant and were at risk for “primary” infection. This is in contrast to the experience of Shields et al[7] with bone marrow recipients, among whom a high percentage were found to have preexisting neutralizing antibodies suggesting that these infections may be due to “reactivation” of a latent recipient infection. The lower incidence of seropositivity in our recipients is probably related to the majority of recipients being less than 5 years of age. Our experience also suggests that A V hepatitis may have greater propensity toward massive liver necrosis in recipients that do not have preexisting antibodies.

The case fatality rate for A V hepatitis in our series was high at 30%. The absence of any known effective chemotherapeutic agent makes treatment of A V hepatitis, other than lowering of immunosuppressive therapy difficult.

There have been sporadic reports of the use of immunoglobulin and thymic humoral factor with favorable results.[8, 9] Massive liver necrosis and fulminant liver failure require retransplantation. The absence of recurrence of A V hepatitis in the second allograft in the 2 survivors of retransplantation suggests that this may be a prudent policy.

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Table 1

Results of Antibody Titers and Clinical Course of 10 Children With AV Hepatitis

Patient No.	Serotype	Donor	Recipient	Result
1	5	NA	NA	Retransplanted; recovered
2	5	NA	Pos 1:64	Recovered
3	5	Neg 1:<2	Pos 1:6	Recovered
4	5	NA	Pos 1:4	Died
5	5	Pos 1:4	Neg 1:<2	Died during retransplant
6	5	Pos 1:4	Neg 1:2	Retransplanted; recovered
7	1	Pos 1:4	Neg 1:<2	Died during retransplant
8	1	NA	Neg 1:<2	Died
9	2	Pos 1:32	Neg 1:<2	Died during retransplant
10	5	Pos 1:4	Neg 1:<2	Recovered

NA = serum not available.