

Brief Report

CORRECTION OF THE HYPER-IgM SYNDROME AFTER LIVER AND BONE MARROW TRANSPLANTATION

NEDIM HADŽIĆ, M.D., ANTONIO PAGLIUCA, M.D.,
 MOHAMED RELA, M.D., BERNARD PORTMANN, M.D.,
 ALISON JONES, PH.D., PAUL VEYS, M.D.,
 NIGEL D. HEATON, M.D., GHULAM J. MUFTI, M.D.,
 AND GIORGINA MIELI-VERGANI, PH.D.

THE hyper-IgM syndrome, a rare form of combined primary immunodeficiency, is characterized by neutropenia and defective B-cell isotype switching, which results in elevated or normal levels of serum IgM and low levels of serum IgG, IgA, and IgE.¹ Clinically, patients with the syndrome have serious pyogenic infections caused by encapsulated bacteria, suggestive of the presence of humoral immunodeficiency. They are also susceptible to infections with intracellular pathogens such as *Pneumocystis carinii*, *Cryptosporidium parvum*, and leishmania, because of a possible defect in cellular immunity.^{2,3}

The inheritance is usually X-linked, but autosomal recessive and autosomal dominant forms have also been documented.⁴ The X-linked form is caused by defects in the gene encoding the CD40 ligand, which is located at Xq26.3–27.⁵ Seventy-five different mutations have been described.⁶ Early treatment with intravenous immune globulin and antibiotic prophylaxis have reduced the incidence of life-threatening infections and improved the growth of children with the syndrome but have failed to affect the incidence of hepatic and hematologic complications, including cancer.³ It is estimated that only 20 percent of patients will reach the third decade of life and that 75 percent of these patients will have liver complications.³ Various gastrointestinal cancers, including cholangiocarcinoma, hepatocellular carcinoma, and adenocarcinoma, were recently described in a cohort of boys with the hyper-IgM syndrome and cholangiopathy.⁷ In that study 70 percent of the boys who were systematically screened for infection had *C. parvum* in-

fection, and all had clinically significant chronic liver disease.⁷ In another study, three patients with the hyper-IgM syndrome died of progressive cholangiopathy associated with recurrent cryptosporidium infection within 25 months after otherwise successful liver transplantation.⁸

Conventional allogeneic bone marrow transplantation has been reported to cure X-linked hyper-IgM syndrome,⁹ but it should be performed before the onset of severe liver disease, since conditioning regimens can be highly hepatotoxic. Recently, less toxic, nonmyeloablative conditioning regimens have been reported to achieve a similar degree of stem-cell engraftment with a reduced rate of complications.^{10,11}

We performed a cadaveric orthotopic liver transplantation together with nonmyeloablative bone marrow transplantation from a matched, unrelated donor in an 18-year-old man with end-stage chronic liver disease associated with the X-linked hyper-IgM syndrome.

CASE REPORT

A three-year-old boy was given a diagnosis of the hyper-IgM syndrome after his younger brother died of *P. carinii* pneumonitis. When he was five years old, the results of liver-function tests were abnormal. A liver biopsy, performed when he was 10, suggested the presence of sclerosing cholangitis. Two years later, endoscopic retrograde cholangiopancreatography showed extrahepatic and intrahepatic cholangiopathy and grade 2 esophageal varices. *Pseudomonas* and *Streptococcus mitis* were isolated from bile culture. Abdominal ultrasonography showed a heterogeneous liver with a nodular margin and a large regenerative nodule that was 5.7 cm in diameter. The spleen was enlarged (17.5 cm). Bone marrow transplantation was considered, but an extensive search failed to identify an HLA-matched donor.

By the age of 18, the patient had had several episodes of hematemesis requiring sclerotherapy and banding of esophageal varices. He became bedridden, with fatigue, gross ascites, spontaneous bleeding, and intractable pruritus. He was treated with immune globulin, albumin, diuretics, vitamin K, ursodiol, and broad-spectrum intravenous antibiotics and received oral antimicrobial prophylaxis with trimethoprim-sulfamethoxazole and paromomycin. His laboratory values were as follows: international normalized prothrombin ratio, 1.96; hemoglobin, 6.2 g per liter; white-cell count, 4040 per cubic millimeter; platelets, 33,000 per cubic millimeter; bilirubin, 32.7 mg per deciliter (559 μ mol per liter); aspartate aminotransferase, 240 U per liter; γ -glutamyltransferase, 30 U per liter; alkaline phosphatase, 114 U per liter; creatinine, 1.9 mg per deciliter (168 μ mol per liter); albumin, 1.8 g per deciliter; and alpha-fetoprotein, 4.5 ng per milliliter (normal level, <7.9). The patient was referred for liver transplantation.

METHODS

Blood samples were obtained from the patient, and activated T cells were assessed for the CD40 ligand by staining with CD40 human IgG1 fusion protein (CD40Fc), a monoclonal antibody to the CD40 ligand. Labeled cells were analyzed on a FACScan (Becton Dickinson, Mountain View, Calif.) with the use of Lysis-2 software. Single-strand conformation polymorphism analysis of individual exons of the CD40 ligand gene was performed¹² and identified an altered band in exon 5. Sequencing confirmed a deletion of 4 bp at position 465 (resulting in a change from GAGCA to C) in the patient and his mother.

In view of the risk of recurrence of life-threatening cholangiopathy after liver transplantation in patients with primary immunodeficiencies,⁸ isolated liver transplantation was not considered.

From the Departments of Child Health (N.H., G.M.-V.), Haematological Medicine (A.P., G.J.M.), and Transplant Surgery (M.R., N.D.H.) and the Institute of Liver Studies (B.P.), King's College Hospital; and the Host Defense Unit, Hospital for Sick Children (A.J., P.V.) — both in London. Address reprint requests to Dr. Hadžić at the Department of Child Health, King's College Hospital, Denmark Hill, London SE5 9RJ, United Kingdom, or at nedim.hadzic@kcl.ac.uk.

©2000, Massachusetts Medical Society.

After a worldwide, media-supported search, a suitable bone marrow donor was identified. The liver transplantation was scheduled to be followed by nonmyeloablative bone marrow transplantation five weeks later, since we assumed that this schedule would allow enough time for normal liver-graft function to be established between procedures. Written informed consent was obtained from the patient.

Blood products received during and after surgery were negative for cytomegalovirus and had been irradiated. Twice each week, an early nuclear fluorescence antigen test for cytomegalovirus was performed on throat-swab, urine, and blood specimens, and stool specimens were checked for cryptosporidium.

RESULTS

Liver Transplantation

In October 1998, two weeks after admission, the patient received a left lateral segment of a liver graft from a cadaveric male donor. The graft was matched for blood group (type O) and was negative for cytomegalovirus. The patient's HLA haplotype was A24,A68, B14,B39,C0702,C0802,DRB1 0404,DRB1 1303, DQB1 0301,DQB1 0302, and that of the donor was A1,A11,B8,B39,Cw7,Cw12,DR4,DR17,DQ2,DQ3. The explanted liver was cirrhotic and collapsed, with scattered prominent areas of hypertrophy (Fig. 1). Biliary cirrhosis, severe cholestasis, and advanced ductopenia were found on microscopical examination. The gallbladder showed areas of papillary hyperplasia with foci of epithelial dysplasia. No pathogens, including cryptosporidium, were identified.

Immunosuppression with tacrolimus (0.1 mg per kilogram of body weight per day) and methylprednisolone (1 mg per kilogram per day) was commenced

on the first day after liver transplantation. Prophylactic antimicrobial treatment included intravenous paromomycin (15 mg per kilogram per day) and oral paromomycin (30 mg per kilogram per day), broad-spectrum antibiotics, liposomal amphotericin B (1 mg per kilogram per day), ganciclovir (10 mg per kilogram per day), and nebulized pentamidine (300 mg every two weeks). Immune globulin (0.5 mg per kilogram) was given once a week.

Candida albicans was identified in the sputum three days after liver transplantation. Postoperative problems included a small perihepatic hematoma, consolidation in the left lower lobe of the lung, and the isolation of vancomycin-sensitive *Staphylococcus epidermidis* from blood cultures and the tip of the central catheter. The dose of tacrolimus was adjusted to achieve serum levels between 3 and 6 μg per liter. By day 10, the graft was functioning satisfactorily, with no evidence of acute cellular rejection. On day 26, liver biopsy was performed and showed only a minor degree of nonspecific portal inflammation.

Bone Marrow Transplantation

Conditioning for bone marrow transplantation was started 22 days after liver transplantation. Intravenous fludarabine (30 mg per square meter of body-surface area) was given for the first five days, and intravenous melphalan on the following day. Because of a low glomerular filtration rate (58 ml per minute), the dose of melphalan was 100 mg per square meter.

Bone marrow transplantation was performed in No-



Figure 1. The Cirrhotic Liver of the Patient. There are alternating areas of macronodular hypertrophy and collapse.

ember 1998, 34 days after liver transplantation. The female bone marrow donor was positive for cytomegalovirus, as was the recipient. The marrow was matched for blood group and for class I and class II major-histocompatibility-complex loci: HLA-A, B, C, DRB1, and DQB1 (a 10-antigen match). A total of 1.31×10^8 bone marrow mononuclear cells per kilogram, 6.55×10^6 CD34+ cells per kilogram, and 16.8×10^4 granulocyte-macrophage colony-forming units per kilogram were infused. As prophylaxis against graft-versus-host disease, the patient was given rabbit anti-T-lymphocyte globulin (one vial per 10 kg) from day 32 through day 36 and methylprednisolone (1 mg per kilogram per day) beginning on day 37. The immunosuppressive treatment with tacrolimus was changed to cyclosporine on day 33, with levels titrated to achieve a trough level of 150 μ g per liter, according to our protocol for bone marrow transplantation. To minimize myelosuppression, nebulized pentamidine was administered monthly, and ganciclovir was replaced by acyclovir (500 mg per square meter per day). Treatment with granulocyte colony-stimulating factor (5 μ g per kilogram per day) was commenced on day 42 and continued until engraftment. In the second week after bone marrow transplantation, a rash appeared (indicating mild graft-versus-host disease) and culture-negative diarrhea developed; both responded to an increased dose of prednisolone (2 mg per kilogram per day).

On day 52, the patient had an isolated grand mal seizure, during which he sustained intracapsular fracture of both femoral necks as a result of poor bone mineralization. Magnetic resonance angiography demonstrated a nonenhancing low-density lesion in the right parietal lobe, possibly due to vasculitis, which resolved within one week. Cultures of cerebrospinal fluid for bacteria, viruses, and mycobacteria were negative. The electroencephalogram was normal. The patient was treated with phenytoin and carbamazepine until day 180.

Neutrophil engraftment, with a peripheral count of more than 500 per cubic millimeter, occurred on day 47. The next day, the patient's platelet count exceeded 20,000 per cubic millimeter. Analysis of variable-number tandem repeats in whole-blood DNA (for von Willebrand factor at intron 40)¹³ showed that there was full donor chimerism on day 52. This finding was confirmed in analysis of bone marrow on day 95. Flow-cytometric analysis of the expression of the CD40 ligand on T cells showed no expression on day 64, but three weeks later 35 percent of cells were positive, indicating adequate expression. On day 248, standard cytogenetic analysis confirmed the presence of a female phenotype in all cells analyzed. In August 1999, nine months after bone marrow transplantation, the level of expression of the CD40 ligand was 55 percent in our patient and 54 percent in a control subject.

In 1995 and 1996, the patient's IgM levels had exceeded 3.0 g per liter (normal range, 0.5 to 1.9). In June 1999, they were 0.53 and 0.65 g per liter on two separate occasions. In August 1999, the level was 2.09 g per liter.

On day 76 abnormal results on liver-function tests prompted a liver biopsy, which demonstrated mild cholangiolitic changes, with no evidence of cellular rejection or hepatitis. To minimize the dose of corticosteroids, immunosuppressive treatment with cyclosporine was changed back to tacrolimus, with the dose titrated to achieve trough levels of 8 to 10 μ g per liter. The aspartate aminotransferase values returned to normal, but levels of γ -glutamyltransferase remained elevated (469 U per liter), prompting another liver biopsy three weeks later. The biopsy revealed a mild focal injury of the bile ducts, with minimal edema of the portal tract and no evidence of cellular rejection or hepatitis. Possible explanations included mild graft-versus-host disease or early pathologic changes affecting the biliary tree. On day 118, the patient was discharged in good clinical condition.

In December 1999, 14 months after liver transplantation and 13 months after bone marrow transplantation, the patient was in excellent health, with satisfactory function of both grafts, a hemoglobin level of 12.3 g per liter, 6500 white cells per cubic millimeter, 2740 neutrophils per cubic millimeter, 156,000 platelets per cubic millimeter, an international normalized prothrombin ratio of 1.0, a bilirubin level of 0.6 mg per deciliter (10 μ mol per liter), an aspartate aminotransferase level of 39 U per liter, a γ -glutamyltransferase level of 52 U per liter, and an albumin level of 3.5 g per deciliter.

DISCUSSION

Patients with primary immunodeficiencies have traditionally been considered unsuitable candidates for elective solid-organ transplantation because of the risks associated with lifelong immunosuppression, as well as the risk of recurrence of end-organ disease. Stem-cell replacement is an established treatment for a variety of hematologic, immunologic, metabolic, and malignant conditions.¹⁴ In some patients with primary immunodeficiencies, progressive cholangiopathy develops, possibly related to chronic opportunistic infections.^{7,15} Bone marrow transplantation can be followed by a number of immediate and late hepatic problems, including veno-occlusive disease, graft-versus-host disease, and drug-induced hepatotoxicity, particularly in patients whose liver function is already suboptimal.¹⁴ Lifesaving liver transplantation has been performed in adults with severe graft-versus-host disease and veno-occlusive disease after bone marrow transplantation.¹⁶ In our patient, bone marrow transplantation was not considered to be an appropriate initial treatment because of his severely compromised liver function. Simultaneous liver and bone marrow

transplantation involving hepatic tissue and bone marrow from a living related haploidentical donor was not possible because of the lack of an appropriate donor.

To promote stem-cell engraftment, bone marrow transplantation is preceded by a conditioning regimen that conventionally includes myeloablative chemotherapy.¹⁴ The standard approach involves total-body irradiation and treatment with busulfan and commonly leads to severe complications in patients with substantial organ damage before transplantation.^{10,11} It has been argued that milder, nonmyeloablative regimens would be less toxic but would still allow donor stem cells to compete successfully for space in the recipient's bone marrow. Patients with inadequate levels of donor chimerism could receive repeated infusions of donor lymphocytes.^{10,11} The use of a combination of cadaveric liver transplantation and nonmyeloablative bone marrow transplantation could increase the risk of graft-versus-host disease because of the presence of different sets of HLA phenotypes and the possibility of mismatching for minor antigenic determinants. Our patient had mild clinical manifestations of skin and, possibly, intestinal graft-versus-host disease, which were controlled by an increased dose of corticosteroids. It is possible that the changes in the bile ducts on liver biopsy that were associated with elevated γ -glutamyltransferase levels also represented a mild graft-versus-host reaction.

CD40 is a 50-kd surface molecule expressed on B cells, monocytes, dendritic cells, epithelial cells, and endothelial cells.^{1,17} The CD40 ligand is expressed mainly on the surface of activated CD4⁺ T cells. The interaction of CD40 and its ligand activates auxiliary pathways of the immune response in conjunction with costimulatory molecules such as B7-1 (CD80) and B7-2 (CD86) on antigen-presenting cells and their receptors, CD28 and CTLA-4, on T cells.¹⁷ In our patient, the absence of rejection of the liver transplant may have been due in part to the inefficiency of his immune system before bone marrow engraftment.

Chronic *C. parvum* infection is common in patients with primary and secondary immunodeficiencies.^{7,15,18,19} Methods of detection, including standard stool and bile surveillance, enzyme-linked immunosorbent assays, and polymerase-chain-reaction-based techniques, all have very limited sensitivity.⁷ Furthermore, therapeutic approaches, including the use of paromomycin,²⁰ azithromycin,²¹ letrozolil,²² and interleukin-2,²³ are of unproved value. Microsporidium may also have a pathogenic role in the development of cholangitis in patients with human immunodeficiency virus infection.²⁴ Chronic cryptosporidium infection of the bile ducts was proposed as a possible contributory factor to the development of cancers.⁷ The presence of dysplastic changes in the biliary epithelium of the explanted liver in our patient, though he remained persistently cryptosporidium-negative, is consistent with the observation by Hayward et al.

of an increased incidence of gastrointestinal cancers in boys with the hyper-IgM syndrome.⁷ Some of the reported tumors express CD40, and the development of an efficient method of surveillance for tumors that express CD40 may lead to earlier detection.⁷

As our findings demonstrate, the combination of liver and nonmyeloablative bone marrow transplantation is a promising treatment approach for patients with end-stage liver disease related to CD40 ligand deficiency. Bone marrow transplantation should be performed as soon as liver-graft function is satisfactory in order to promote immunocompetence and prevent reinfection with opportunistic pathogens. Timely recognition of liver impairment, the use of anti-cryptosporidium prophylaxis, and early bone marrow transplantation, when a related, HLA-matched donor is available, may arrest the progression of serious complications. Combined correction of the primary immune defect and the secondary liver complications could then be reserved for patients who already have advanced liver disease at presentation.

We are indebted to Dr. Edvard T. Davies, Department of Immunology, King's College Hospital, London, for performing flow cytometric analysis of CD40 ligand expression.

REFERENCES

1. Notarangelo LD, Duse M, Ugazio AG. Immunodeficiency with hyper-IgM (HIM). *Immunodef Rev* 1992;3:101-21.
2. Grewal IS, Borrow P, Pamer EG, Oldstone MBA, Flavell RA. The CD40-CD154 system in anti-infective host defence. *Curr Opin Immunol* 1997;9:491-7.
3. Levy J, Espanol-Boren T, Thomas C, et al. Clinical spectrum of X-linked hyper-IgM syndrome. *J Pediatr* 1997;131:47-54.
4. Primary immunodeficiency diseases: report of a WHO scientific group. *Clin Exp Immunol* 1995;99:Suppl 1:2-24.
5. Allen RC, Armitage RJ, Conley ME, et al. CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome. *Science* 1993;259:990-3.
6. Notarangelo LD, Peitsch MC. CD401base: a database of CD40L gene mutations causing X-linked hyper-IgM syndrome. *Immunol Today* 1996;17:511-6.
7. Hayward AR, Levy J, Facchetti F, et al. Cholangiopathy and tumors of the pancreas, liver, and biliary tree in boys with X-linked immunodeficiency with hyper-IgM. *J Immunol* 1997;158:977-83.
8. Martinez Ibanez V, Espanol T, Matamoros N, et al. Relapse of sclerosing cholangitis after liver transplant in patients with hyper-IgM syndrome. *Transplant Proc* 1997;29:432-3.
9. Thomas C, de Saint Basile G, Le Deist F, et al. Correction of X-linked hyper-IgM syndrome by allogeneic bone marrow transplantation. *N Engl J Med* 1995;333:426-9.
10. Giralt S, Estey E, Albitar M, et al. Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus-leukemia without myeloablative therapy. *Blood* 1997;89:4531-6.
11. Slavin S, Nagler A, Naparstek E, et al. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. *Blood* 1998;91:756-63.
12. Villa A, Notarangelo LD, Di Santo JP, et al. Organization of the human CD40L gene: implications for molecular defects in X-chromosome-linked hyper-IgM syndrome and prenatal diagnosis. *Proc Natl Acad Sci U S A* 1994;91:2110-4.
13. Mancuso DJ, Tuley EA, Westfield LA, et al. Structure of the gene for human Willebrand factor. *J Biol Chem* 1989;264:19514-27.
14. Kapoor N, Crooks G, Kohn DB, Parkman R. Hematopoietic stem cell transplantation for primary lymphoid immunodeficiencies. *Semin Hematol* 1998;35:346-53.

15. Hadzic N, Heaton ND, Francavilla R, Davies G, Jones A, Mieli-Vergani G. Paediatric sclerosing cholangitis associated with primary immunodeficiency. *Hepatology* 1998;28:Suppl:446A. abstract.
16. Rosen HR, Martin P, Schiller GJ, et al. Orthotopic liver transplantation for bone marrow transplant-associated veno-occlusive disease and graft-versus-host disease of the liver. *Liver Transpl Surg* 1996;2:225-32.
17. Fuleihan RL. The X-linked hyperimmunoglobulin M syndrome. *Semin Hematol* 1998;35:321-31.
18. Davis JJ, Heyman MB, Ferrell L, Kerner J, Kerlan R Jr, Thaler MM. Sclerosing cholangitis associated with chronic Cryptosporidiosis in a child with a congenital immunodeficiency disorder. *Am J Gastroenterol* 1987; 82:1196-202.
19. Petersen C. Cryptosporidiosis in patients infected with the human immunodeficiency virus. *Clin Infect Dis* 1992;15:903-9.
20. Armitage K, Flanigan T, Carey J, et al. Treatment of cryptosporidiosis with paromomycin: a report of five cases. *Arch Intern Med* 1992;152: 2497-9.
21. Hicks P, Zwiener RJ, Squires J, Savell V. Azithromycin therapy for *Cryptosporidium parvum* infection in four children infected with human immunodeficiency virus. *J Pediatr* 1996;129:297-300.
22. Blanshard C, Shanson DC, Gazzard BG. Pilot studies of azithromycin, letrazuril and paromomycin in the treatment of cryptosporidiosis. *Int J STD AIDS* 1997;8:124-9.
23. Nachbar D, Kropshofer G, Feichtinger H, Allerberger F, Niederwieser D. Cryptosporidiosis after CD34-selected autologous peripheral blood stem cell transplantation (PBSCT): treatment with paromomycin, azithromycin and recombinant human interleukin-2. *Bone Marrow Transplant* 1997;19:1261-3.
24. Pol S, Romana CA, Richard S, et al. Microsporidia infection in patients with the human immunodeficiency virus and unexplained cholangitis. *N Engl J Med* 1993;328:95-9.