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A Case of Advanced Primary Biliary Cirrhosis Treated with Granulocyte and Monocyte Apheresis[1]

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Abstract: Generally, the most effective treatment for advanced primary biliary cirrhosis (PBC) is liver transplantation, but adjunct therapies are needed. We report here a first case of advanced PBC treated with a new immunotherapy, granulocyte and monocyte apheresis (GCAP). A column (Adacolumn, Japan Immunoresearch Laboratory Takasaki, Japan) was filled with cellulose acetate beads to selectively adsorb granulocytes and monocytes/macrophages. A 49-year-old woman was diagnosed with PBC in 1987. In June 2001, steroid pulse therapy and adjuvant fresh frozen plasma was given for moderate jaundice but without success. In July, as total bilirubin rapidly increased, treatment with GCAP was started and succeeded in suppressing the rapid deterioration of total bilirubin (value changes after each of four applications: 15.4→14.0, 27.2→25.1, 25.8→24.0, 25.7→23.7 mg/dL) and improving prothrombin time (16.4→14.5 s). Although GCAP therapy did not prevent a fatal outcome, it suppressed rapid deterioration of jaundice and increased quality of life for a month. **Key Words:** ELAM-1—Granulocyte and monocyte apheresis—Immunotherapy—Primary biliary cirrhosis—Selectin.

Primary biliary cirrhosis (PBC) is presumed to be an autoimmune liver disease predominantly affecting middle-aged women (1). It is characterized by disease-specific, non-organ-specific antimitochondrial (AMA) and/or antinuclear (ANA) autoantibodies and by autoimmune genetic susceptibility, with the presence of susceptibility alleles on the major histocompatibility complex (MHC) on chromosome 6 (1,2). It is a chronic cholestatic disease, characterized by granulomatous destruction of the interlobular and septal bile ducts and development of portal and periportal inflammation, subsequent fibrosis, and eventually cirrhosis. Advanced PBC is marked by rapid deterioration of jaundice and several complications related to portal hypertension such as variceal bleeding, ascites and hepatic encephalopathy (1). The most effective treatment for advanced PBC is orthotopic liver transplantation (1,3).

However, because of a shortage of suitable donors, alternative treatments which effectively prolong life are needed. While ursodeoxycholic acid (UDCA) and several immunosuppressive agents have proven to be effective in patients with early PBC, tests using cases with advanced PBC have demonstrated no benefit (1,3,4). While plasma exchange and/or plasma bilirubin adsorption therapy has also been employed with improvement in pruritis and hypercholesterolemia (5,6), the amelioration of jaundice is only temporary and effects on survival have been limited (5,6). One new immunotherapy, granulocyte and monocyte apheresis employing cellulose acetate beads for cell adsorption (GCAP), has recently been introduced for treatment of active ulcerative colitis (7) and rheumatoid arthritis (8,9) in Japan. We report a first case of advanced PBC treated with GCAP.

CASE REPORT

In 1987, a 49-year-old woman was diagnosed with antimitochondrial antibody (AMA)-negative PBC (stage 2) complicated by Sjögren's syndrome and confirmed by a wedge biopsy of the liver (Fig. 1). She visited our office in May, 1991, and reported pruritus. The patient had no family history of liver or autoimmune diseases, and no past history of alcohol abuse or blood transfusion. She had been treated for chole-

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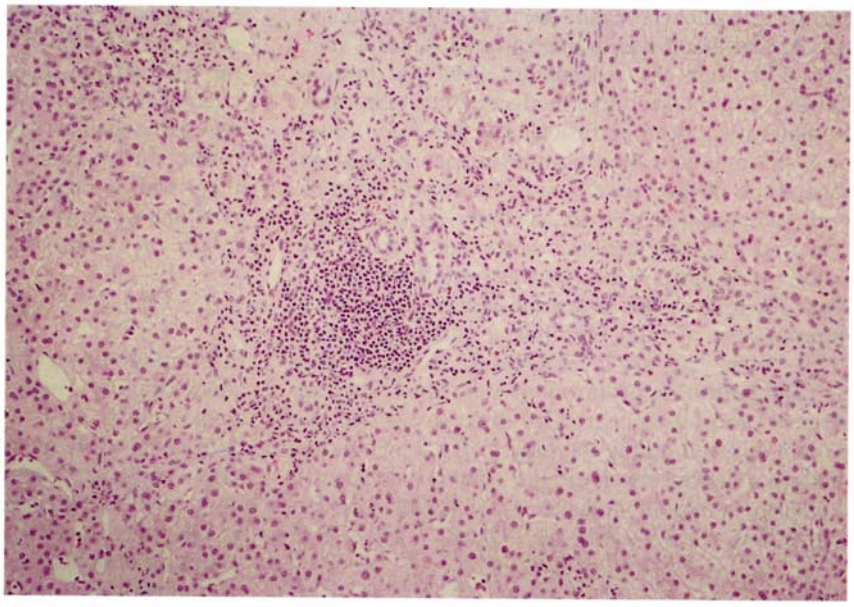

FIG. 1. Wedge biopsy of the liver. Note the enlarged portal tract containing proliferated ductules and a lymphoid aggregate. Destructive and degenerative changes of interlobular bile ducts are also evident. The boundary of the portal tract is poorly defined because of piecemeal necrosis of fibrotic type. These histological features are compatible with chronic nonsuppurative destructive cholangitis, PBC (stage 2). H&E, ×125. (November 11, 1987).

cystitis at 14 years of age. Physical examination revealed a small operative scar from the wedge biopsy of the liver at the right upper quadrant of abdomen. Laboratory data were as follows: total bilirubin (TB), 1.9 mg/dL; direct bilirubin (DB), 1.2 mg/dL; aspartate aminotransferase (AST), 63 IU/L; alanine aminotransferase (ALT), 36 IU/L; alkaline phospatase, 615 IU/L (normal <223); lactic dehydrogenase, 333 IU/L; gamma-glutamyl-transpeptidase, 133 IU/L; leucine aminopeptidase, 99 IU/L; antimitochondrial antibodies–; antinuclear antibodies + (centromere x1280); anti-DNA antibodies–; anti-RNP antibodies–; anti-SSA antibodies–; anti-SSB antibodies–; antismooth muscle antibodies–; immunoglobulin-G, 1881 mg/dL; immunoglobulin-M, 406 mg/dL; C_3, 69 mg/dL; C_4, 17.7 mg/fl; CH50, 34 U/mL; immune complex (C_{1q}), 1 μg/mL; HBsAg–; HCV—(3rd generation, Chiron, USA); white blood cells, 5200/mm^3; red blood cells, 353×10^4/mm^3; hemoglobin, 11.4 g/dL; platelets, 7.9×10^4/mm^3; its prothrombin time, 11.9 s (normal <12.2). Mild esophageal varices were detected by endoscopy. From 1987, UDCA (600 mg/day) was administered and pruritus fluctuated from a moderate degree to very mild, and completely disappeared in 1996 with adjuvant cholestyramine. The total administration period of UDCA was 14 years.

On June 1, 2001, as total bilirubin increased to 4.4 mg/dL, steroid pulse therapy (methylprednisolone 0.5 g/day × 3) was given four times, and adjuvant fresh frozen plasma was also applied but without success. On July 10, 2001, as the value was elevated to 15.4 mg/dL and ascites appeared (Fig. 2), registration for cadaveric liver transplantation was completed. However, as the number of cadaveric donors is very low in Japan, treatment to effectively prolong life was needed. Plasma exchange and/or plasma bilirubin adsorption therapy for advanced PBC has not proven to effectively prolong life (5,6). The new immunotherapy, GCAP, was therefore started instead. For this purpose, a column (Adacolumn, Japan Immunoresearch Laboratory Takasaki, Japan) was filled with 220 g cellulose acetate beads (about 35 000 pieces) of 2 mm in diameter to selectively

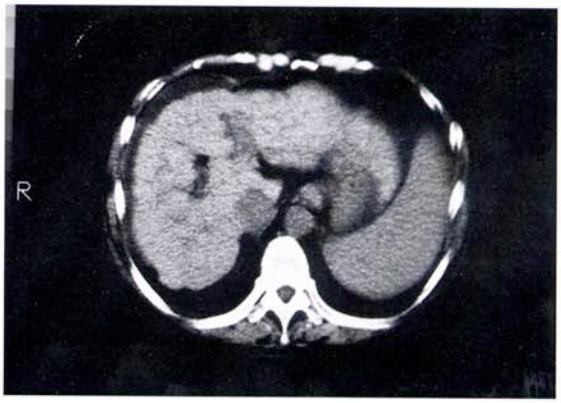

FIG. 2. Computed tomography of the liver demonstrates characteristic changes of cirrhosis. The liver is rather small and has a nodular contour with surrounding ascites and the spleen is enlarged. (July 9, 2001).

adsorb granulocytes and monocytes/macrophages (8). Patient blood was drained from a femoral vein, passed through the Adacolumn from the bottom upward, and returned to a median cubital vein using a pump (direct hemoperfusion). The flow rate was set at 30 mL/min, and the duration of each circulation session was 60 min. Total blood volume processed on each occasion was 1800 mL. Samples for liver function and cytokine analyzes were taken from the inflow route. GCAP therapy was performed once a week for four weeks, and succeeded in suppressing deterioration of total bilirubin at each application (15.4→14.0, 27.2→25.1, 25.8→24.0, 25.7→23.7 mg/dL) and improving prothrombin time (16.4→14.5 s). Ascites almost disappeared for three weeks and the patient felt well during the period (Fig. 3). Cytokine analysis revealed levels of the soluble endothelial leukocyte adhesion molecule-1 (sELAM-1) to be suppressed (68.4→58, 81.2→65.8, 106→98.5, 69.8→68.8 ng/mL) (normal <80.5) in accordance with the reduction in bilirubin (Fig. 3). Two days after the fifth session of GCAP, the patient experienced complications with clouding of consciousness caused by psychogenic reactions. GCAP therapy was resumed after an interval of 2 weeks. In spite of treatment with another six sessions (twice a week), she continued a downhill course and expired of hepatic failure on October 5, 2001.

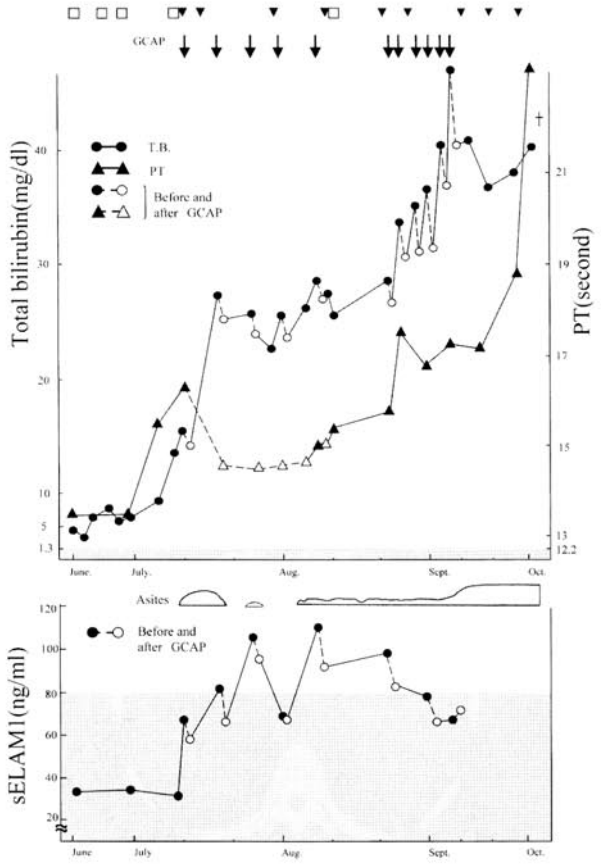

FIG. 3. Clinical course. TB, total bilirubin; DB, direct bilirubin; PT, prothrombin time; sELAM-1, soluble endothelial leukocyte adhesion molecule-1; , normal value range; □, steroid pulse therapy; ▼, infusion of fresh frozen plasma; ▼, granulocyte and monocyte apheresis (GCAP);●---○, ■---□, before and after GCAP.

DISCUSSION

Primary biliary cirrhosis is a disease in which autoimmunity is believed to play an important pathogenic role (1,2). It is characterized by damage to intrahepatic bile ducts and surrounding hepatocytes due to infiltrating lymphocytes and other inflammatory cells (1). At present, the most attractive theory is the concept of molecular mimicry, in which infiltrating T lymphocytes (T cells) recognize host protein mimicking foreign molecules (10). This reactivity initiates an autoimmune cascade leading to destruction of bile ducts. Cytokines produced by T lymphocytes and other cells have also been postulated to be involved, with mRNAs for IL-2, IL-5, IL-6, TGF-β and IFN-γ detected in the liver (11), and IFN-γ mRNA-positive cells found primarily around damaged bile ducts (12).

The new immunotherapy, GCAP, has been used for the treatment of active ulcerative colitis (7) and rheumatoid arthritis (8,9) in Japan, and succeeded in improving clinical symptoms. The column mainly adsorbs granulocytes and monocytes but not lymphocytes, especially T cells (8). The main aim in treatment of ulcerative colitis and rheumatoid arthritis is their depletion (7–9). However, as T cells interact with the various types of cells adsorbed by beads, and pass through the shower of cytokines and gas mediators (O_2^-) released from adherent cells, their phenotype changes (8) and their cytokine (IFN-γ) production decreases (13).

Based on the pathogenesis of PBC, and the mechanism of GCAP, we thought that this new immunotherapy might be a logical approach to reduce the function of T cells and ameliorate the deranged immune status. In fact, the present clinical study revealed a number of changes after GCAP therapy (Fig. 3). First, the rapid deterioration due to jaundice was suppressed for the first four sessions, with moderate improvement of total bilirubin after each (15.4→14.0, 27.2→25.1, 25.8→24.0, 25.7→23.7 mg/dL) and of direct bilirubin(11.9→11.3, 21.4→20.0, 21.0→18.2, 19.0→17.2 mg/dL). Second, prothrombin time improved moderately (16.4→14.5 s) and this

persisted for 4 weeks. However, no significant improvement in other liver function tests, such as for AST, ALT, albumin, cholinesterase and platelet, was detected.

Cytokine analysis revealed sELAM-1 to gradually increase in accordance with the deterioration of total bilirubin and to be suppressed after GCAP therapy for the first four sessions along with the suppression of bilirubin (Fig. 3). ELAM-1 is exclusively expressed by cytokine (TNF-α and IL-1β)-activated endothelial cells. By interacting with its carbohydrate ligand, it is important in the early adhesion and rolling of neutrophils, monocytes and lymphocytes along the endothelial lining (14) and endothelial expression of ELAM-1 is a hallmark of acute cytokine-mediated inflammation (15). In PBC cases, ELAM-1 is expressed strongly on vascular endothelium, suggesting facilitation of recruitment of lymphocytes around the bile ducts (16). Recently, a soluble form of ELAM-1 (sELAM-1) has been identified (17) with increased levels in a variety of human malignancies, severe sepsis, and autoimmune diseases, including systemic erythematosus, vasculitis (18) and PBC (19,20). It is reported that sELAM-1 levels are significantly higher in patients with late PBC (Stages 3, 4) as compared with early PBC (Stages 1, 2) (20).

In the present case, the fact that standard steroid pulse immunotherapy was given without success, yet GCAP therapy alleviated the deterioration of liver function means some special mechanism must be postulated. During apheresis within the column, a strong inflammatory environment is created following interaction between inflammatory cells and the column carriers, resulting in the release of cytokines (IL-Iβ, IL-6, IL-8 and TNF-α), reactive oxygen species and neutrophil granule enzymes (7–9,21). It has recently been suggested that lipid peroxidation may be involved in the pathogenesis of PBC (22–24). Neutrophil and monocyte functions in the production of oxygen-derived free radicals in PBC have been reported to be elevated, and hepatocellular lipid peroxidation is considered to play a role in hepatic injury in advanced stage PBC (23). In the present case, treatment with GCAP slightly suppressed total cholesterol levels at each session (148→137, 121→112, 97→90, 118→102 mg/dL). The possibility that hepatocellular lipid peroxidation is also suppressed via GCAP clearly warrants further attention. Limited data are available regarding the pathogenesis of jaundice in advanced PBC. The fact that a fatal outcome was not prevented by the present GCAP therapy indicates that not all underlying conditions could be suppressed.

In summary, we report a first case of advanced PBC treated with GCAP. Although a fatal outcome was not prevented, suppression of rapid deterioration was evident. This might be of significance for patients who are waiting for transplant donors. Further cases must now be evaluated.

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