Role of Immunologic Costimulatory Factors in the Pathogenesis of Biliary Atresia

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Background: The authors studied the patterns of expression of immunologic costimulatory molecules (B7-1, B7-2, and CD40) in biliary atresia (BA) patients to confirm any correlation with clinical course/outcome.

Methods: Based on clinical status 2 years postoperatively, 24 BA patients were divided into group I (n = 8, normal liver function), group II (n = 10, anicteric with moderate liver dysfunction), and group III (n = 6, icteric with severe liver dysfunction). Liver biopsies obtained at portoenterostomy and from 6 age-matched controls, were analyzed immunohistochemically using antibodies against B7-1, B7-2, and CD40.

Results: There was no expression of B7-1, B7-2, or CD40 in any control liver specimen. In all BA specimens, B7-1, B7-2, and CD40 were expressed strongly in bile ductules in portal tracts. In groups with liver dysfunction, B7-1, B7-2, and CD40 were expressed strongly on the surfaces of Kupffer and dendritic cells and in hepatocyte cytoplasm. Positive staining cells were significantly fewer in patients with better clinical outcome. B7-1 was found in vascular and sinusoidal endothelial cells only in cases of postoperative portal hypertension.

Conclusions: Costimulatory factors expressed on bile ductules, hepatocytes, and vascular endothelial cells appear to mediate autoimmune processes causing progressive liver fibrosis and portal hypertension in BA.

INDEX WORDS: Biliary atresia, costimulatory factors, fibrosis, portal hypertension.

IN BILIARY ATRESIA (BA), progressive destruction of intrahepatic bile ducts leads to impaired bile secretion and the eventual development of liver cirrhosis according to a process that is incompletely understood, although immune reactions involving T cells and antigens expressed on interlobular bile ducts and hepatocytes are most likely to be implicated.[1,2]

Balanced interaction between antigen-presenting cells (APCs) and lymphocytes is of special importance in the liver, because the healthy liver normally does not mount specific illicit immune responses even though the hepatic immune system is constantly exposed to a large number of antigens that reach the liver via the portal tract.[3] Sufficient immune response depends on efficient T cell activation via costimulatory molecules, and 2 factors are necessary for the activation of T lymphocytes by APCs.[4] One depends on expression of major histocompatibility complex (MHC) class II molecules, which deliver the first signal through their interaction with T cell receptors, and the other depends on expression of B7 family antigens on APCs, which provides the second (costimulatory) signal to T lymphocytes through CD28.[5,6]

The interaction of B7-1 and B7-2 with their counter receptors on T lymphocytes provides a particularly potent costimulatory signal, which amplifies the response of T cells.[7] Although antigen presentation followed by costimulation induces full T cell activation (ie, sufficient immune response), antigen presentation that lacks costimulatory signals results in tolerance or anergy.[8] B7-1 is a 44- to 60-kD member of the immunoglobulin superfamily with a limited expression on professional APCs such as macrophages, dendritic cells, and activated B cells.[9] B7-2 is a 75- to 115-kD cell surface glycoprotein with 25% amino acid homology to B7-1. It also has restricted expression on APCs.[10]

Regulation of costimulation also may involve the CD40 molecule, which is a 45- to 48-kD glycoprotein that can be expressed on a great variety of different cells and interacts with its natural ligand, CD154, on lymphocytes.[11-13]

We studied the expression of costimulatory molecules in BA in an attempt to correlate different expression patterns with clinical course and outcomes.

MATERIALS AND METHODS

We classified 24 long-term follow-up postoperative BA patients (mean age, 12.4 ± 5.4 years; 10 boys, 14 girls) into 3 groups according

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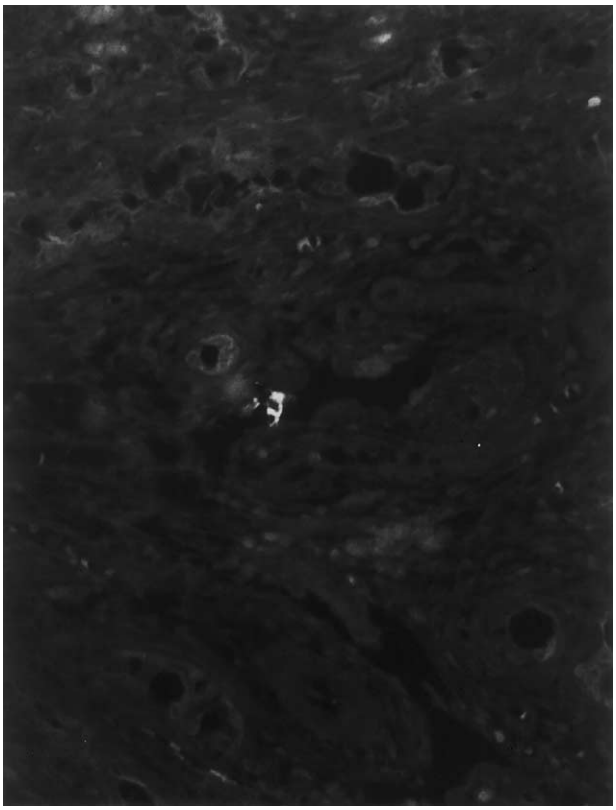

Fig 1. B7-1 in a control liver specimen. No expression of B7-1 is seen in this liver specimen. (Original magnification ×200.)

to their average liver function over the 3 months before the commencement of this study. Liver function was assessed using serum levels of total bilirubin (T-Bil), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and γ-glutamyl transpeptidase (γ-GTP). Group I (n = 8) was jaundice free, had normal liver function (T-Bil, <1.5 mg/dL; GOT, <40 IU/L; GPT, <35 IU/L; γ-GTP, <55 IU/L) and had had no evidence of severe cholangitis or portal hypertension; group II (n = 10) had moderate liver dysfunction (T-Bil, <1.5 mg/dL; GOT, >40 IU/L; GPT, >35 IU/L; γ-GTP,>55 IU/L); and group III (n = 6), the "unfavorable prognosis group" had severe liver dysfunction (T-Bil, >1.5 mg/dL; GOT, >40 IU/L; GPT, >35 IU/L; γ-GTP, >55 IU/L). Five subjects in group II and all subjects in group III had portal hypertension (PH) at the time of assessment. Each BA patient underwent a wedge liver biopsy during portoenterostomy (mean age at surgery, 57.3 days; range, 27 to 83 days). Six histologically normal wedge liver biopsies from 4 patients with choledochal cyst and 2 patients with prolonged jaundice were used as controls (mean age at biopsy, 21.3 months; range, 1.5 to 38 months).

All subjects were investigated after obtaining parental informed consent to participate in this study. This study was approved by the Juntendo University School of Medicine Ethics Committee and complies with the Helsinki Declaration of 1975 (revised 1983).

All specimens were snap-frozen. Frozen 10-μm-thick sections were stained with monoclonal antibody (mAb) B7-1, B7-2, and CD40 immunohistochemistry. Sections were fixed in acetone (4°C) for 10 minutes then incubated for 15 minutes in a solution containing phosphate-buffered saline (PBS), 1% rabbit serum albumin, and 0.3% Tween 20. This solution was used also as a buffer solution for dilution and rinsing.

The primary antibodies used were anti–B7-1 mAb (Becton-Dickinson Inc, San Jose, CA) in a dilution of 1:10; anti–B7-2 mAb (Pharmingen Inc, San Diego, CA) in a dilution of 1:5, and anti–CD40 mAb (Genzyme, Cambridge, MA) in a dilution of 1:10. The secondary antibodies used were antimouse-conjugated Alexa 594 in a dilution of 1:200. In controls, there was immunostaining observed when primary antisera were omitted or replaced with normal/rabbit serum. Floating sections were incubated with each antibody overnight at 4°C on a rotating table. Samples were washed in 3 changes of PBS for 3 hours between subsequent incubations. The sections were observed and reconstructed using a Bio-Rad MRC-1024 invert confocal microscope.

RESULTS

Control Liver Specimens

There was no expression of B7-1, B7-2, or CD40 in any of the control liver specimens (Fig 1).

BA Specimens

In all BA specimens, there was distinct positive staining for B7-1, B7-2, and CD40 seen specifically in the epithelial cells of bile ducts in the portal tracts (Fig 2). In all specimens, B7-1 was detected preferentially in the connective tissue of the liver (Fig 3). In both liver dysfunction (groups II and III), B7-1, B7-2, and CD40 were found to be expressed specifically on the surface of Kupffer cells and dendritic cells and in the cytoplasm of hepatocytes (Fig 4A and B).

However, the number of positive staining cells was significantly lower in patients in the better clinical outcome groups (ie, group II < group III). In addition, in group I, the group with the best prognosis, B7-1, B7-2, and CD40 were detected only in the epithelial cells of bile ducts in the portal tracts. An additional surprising

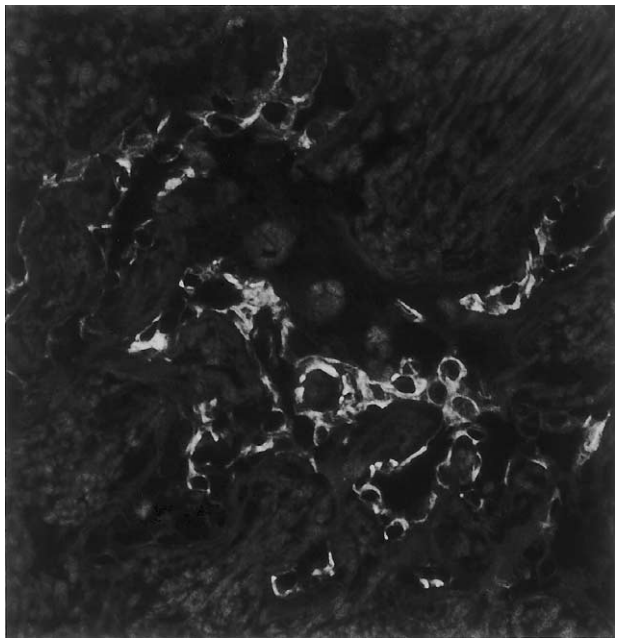

Fig 2. B7-1 in a BA liver specimen. Strong expression of B7-1 is seen in bile duct epithelia cells. (Original magnification ×200.)

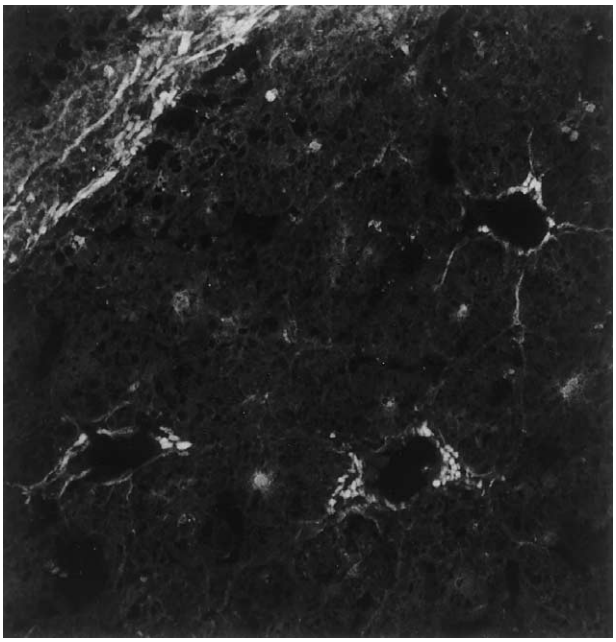

Fig 3. B7-2 in a BA liver specimen. Strong expression of B7-2 is seen in liver connective tissue. (Original magnification ×100.)

finding was that B7-1 was seen in vascular and sinusoidal endothelial cells in all patients who had portal hypertension (PH) postoperatively but not in patients who did not have PH (Fig 5).

DISCUSSION

The Kasai portoenterostomy saved many BA infants from early death, but postoperatively, there is progressive hepatic fibrosis and PH. Investigators identified inflammatory infiltrates in the bile ducts of patients with BA similar to those found in primary sclerosing cholangitis, prompting several researchers to postulate that BA may also be caused by autoimmune phenomena.[2] Some even believe it may be an example of immunologically mediated bile duct injury.[2,14] Certainly, target antigens such as HLA-DR are apparent in postnatal bile ducts in BA, and aberrant expression of adhesion molecules with an infiltration of activated macrophages may cause immune mediated destruction.[2]

We have reported that the relationship between expression of MHC class II antigens on bile ductules or hepatocytes allows those hepatocytes to be better immunogenic targets and CD68+ macrophages of the liver and outcome after portoenterostomy in BA.[14] To date, there have been no data on costimulatory signals in BA, and, in this study, we showed for the first time that B7-1, B7-2, and CD40 are expressed specifically in the epithelial cells of bile ducts in the portal tracts in all BA patients and are absent in the normal liver. These data are in agreement with the findings of a previous report and further support the notion that in BA, bile duct epithelia may be destroyed because of cell mediated immune attack. Thus, the current findings give support to the concept that bile duct epithelial cells in BA may be the target cells for cell-mediated immune mechanisms.

In addition, it would appear that particular patterns of

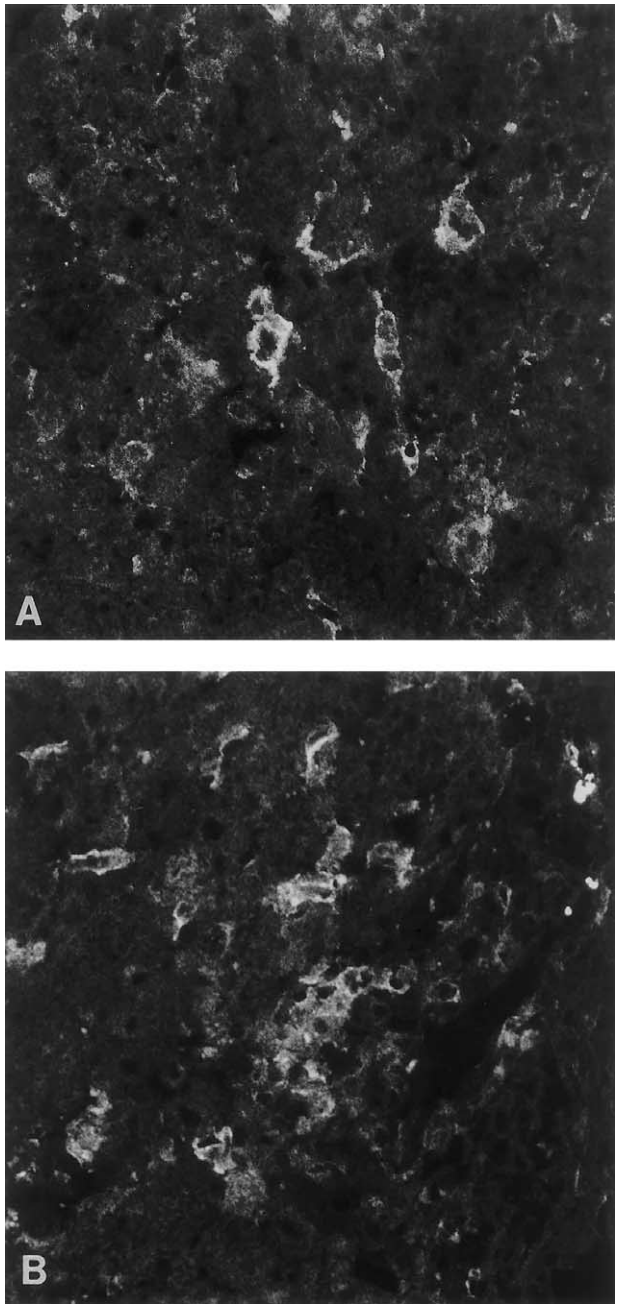

Fig 4. CD40 in BA liver specimen. (A) There are a small number of CD40-positive cells seen on the surface of Kupffer cells, dendritic cells, and in the cytoplasm of hepatocytes in this group II BA liver specimen. (Original magnification ×200.) (B) There are numerous CD40-positive cells seen on the surface of Kupffer cells dendritic cells, and in the cytoplasm of hepatocytes in this group III BA liver specimen. (Original magnification ×200.)

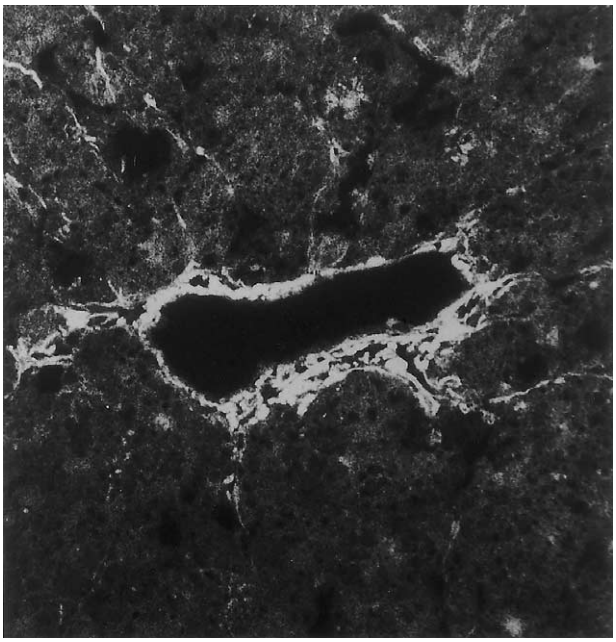

Fig 5. B7-1 in a BA liver specimen. B7-1 is seen in vascular and sinusoidal endothelial cells of all patients with portal hypertension. (Original magnification ×200.)

expression of costimulatory molecules are related to outcome, because we found that in the good prognosis group (group I), costimulatory molecules were found only in the epithelial cells of bile ducts in the portal tracts, whereas in the 2 groups with liver dysfunction (groups II and III), B7-1, B7-2, and CD40 were expressed strongly on the surface of Kupffer cells, dendritic cells, sinusoidal endothelial cells, and in the cytoplasm of hepatocytes. Furthermore, the number of positive cells was significantly lower in patients with better clinical outcome (group II $<$ group III). In other words, the aberrant expression of B7-1, B7-2, and CD40 in biliary epithelium in BA patients and on the membranes of damaged hepatocytes in BA patients with poor prognosis would suggest that the biliary epithelium and hepatocytes in these patients is more susceptible to immune recognition and destruction through some mechanism such as T cell cytotoxicity or locally released cytokine activity.

Also, the interaction of CD40 with its ligand CD154 is believed to be a key step in an upregulation of CD80 (B7-1) and CD86 (B7-2) expression in APCs, which has a strong impact on B cell activation, the initiation of antigen-specific T cell responses, and macrophage maturation.[9] There is circumstantial evidence that expression of CD80 (B7-1)/86 (B7-2) or CD40 conveys potent immunoactivating properties to the cells that express these molecules in BA.[4,15]

An additional surprising finding was the pattern of expression of B7-1 in the vascular and sinusoidal endothelial cells of patients with PH. PH is the result of augmented intrahepatic vascular resistance and increased portal blood flow, and it has been accepted that hepatic stellate cells play a key role in hepatic fibrosis because accumulating evidence from in vitro and in vivo studies suggests that stellate cells are involved in the regulation of liver microcirculation.[16] We believe that immune response mediated through B7-1 might play an important role in the pathogenesis of PH.

Our findings on costimulatory factors may have therapeutic implications because agents that block or prevent costimulatory factors from being expressed could, theoretically, reduce bile duct damage and hepatocyte damage in BA.[17] Further work on this concept is required and will be the subject of further research.

Our observations provide evidence for abnormally upregulated antigen presentation by liver cells in BA as the likely pathway by which immune-mediated processes induce the pathologic changes typical of BA.

In particular, expression of costimulatory molecules is enhanced markedly and may trigger and maintain the inflammatory processes leading to massive hepatocyte damage. Consequently, novel therapeutic strategies targeted at blocking these costimulatory molecules might therefore provide a promising approach to the treatment and prevention of BA.

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Discussion

From the Floor: Many of us use adjuvant steroid therapy in our biliary atresia patients. Based on the evidence you have presented, would it make more sense for us to be using a different immunomodulatory drug?

H. Kobayashi (response): We also use steroids in patients with biliary atresia because there have been some studies that show that aggressive corticosteroid therapy may improve bile drainage and outcome. In particular, expression of costimulatory molecules is markedly enhanced and may trigger and maintain the inflammatory processes leading to hepatocyte and bile duct damage. Of course, in the future, novel therapeutic strategies targeted at blocking these costimulatory molecules might therefore be a promising approach to the treatment and prevention of progressive liver fibrosis in these patients with biliary atresia. However, no one has yet tried these antibodies in humans. I hope this antibody treatment will one day be used and be safe in the patients with biliary atresia.