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Short Communication

Increased Expression of Intercellular Adhesion Molecules in Biliary Atresia

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The expression of the inflammatory adhesion molecules intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and endothelial leukocyte adhesion molecule-1, was studied in six infants with biliary atresia using an immunoperoxidase technique on frozen sections. Controls consisted of five patients with various conditions including total parenteral nutrition-induced cholestasis, choledochal cyst, viral hepatitis, metastatic carcinoma, and thrombotic thrombocytopenic purpura. None of the patients were in liver failure. Bile ducts from the control subjects did not express any of the inflammatory adhesion molecules on ductal epithelium. In marked contrast, all of the biliary atresia specimens demonstrated strong intercellular adhesion molecule-1 expression and occasional vascular cell adhesion molecule-1 staining on epithelial cell membranes of both intra- and extrahepatic ductal structures. Hepatocytes and sinusoidal lining cells including Kupffer cells showed a pattern of intense intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 expression in all specimens with active inflammation that could not differentiate the biliary atresia cases from the control group. Lymphocyte function-associated antigen-1 intensely stained the inflammatory cell infiltrate in the biliary atresia and inflamed control specimens. The strong expression of intercellular adhesion molecule-1 on biliary ductal epithelium in patients with biliary atresia suggests a potential role for this adhesion molecule in the pathogenesis of this devastating neonatal hepatic disorder. (Am J Pathol 1994, 145:263–267)

The pathological hallmark of biliary atresia is an obliterative cholangitis of the extrahepatic bile ducts associated with ductal proliferation that ultimately results in cirrhosis and hepatic failure. The etiology of this destructive process remains unknown, with possibilities ranging from ischemia or viral infection to autoimmune activation of the inflammatory cascade.[1,2] Regardless of the inciting factor, however, the progressive destruction of intra- and extrahepatic bile ducts as a result of the disease process seems to involve an immunologically mediated mechanism of recruitment and activation of various immune effector cells.

The recruitment and maintenance of lymphocyte populations in an area of inflammation is dependent upon cell-cell interactions through three main groups of adhesion receptors: the integrin, the selectin, and the immunoglobulin supergene families.[3] Several studies have recently demonstrated the involvement of adhesion molecules from these groups in the pathogenesis of a number of acute and chronic hepatic inflammatory disorders. Increased expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (ELAM-1) has been shown to occur in primary biliary cirrhosis, primary sclerosing cholangitis, viral hepatitis B, and liver transplant rejection.[4–8] In particular, ICAM-1 expres-

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sion has recently been implicated in the pathogenesis of the destruction of ductal structures associated with primary biliary cirrhosis and primary sclerosing cholangitis.[6]

We hypothesized that the pathogenesis of biliary atresia involved a mechanism of ductal inflammation similar to that reported in primary biliary cirrhosis and sclerosing cholangitis and thus studied the expression and distribution of these intercellular adhesion molecules in this devastating neonatal hepatic disorder.

Materials and Methods

Patients

Eleven liver samples were analyzed: ten were obtained from patients undergoing surgical biopsies for diagnostic purposes, and one was obtained at the time of autopsy. Seven infants ranging in age from 4 to 10 weeks were being evaluated for conjugated hyperbilirubinemia. Six of these infants had biliary atresia, and one had total parenteral nutrition-induced cholestasis. Four patients comprised the control group and included the diagnoses of a choledochal cyst, viral hepatitis, metastatic carcinoma, and thrombotic thrombocytopenia purpura. Diagnoses were made on the basis of clinical, biochemical, and standard histological criteria.

Methods

Each specimen was received fresh and divided into two parts. One part was fixed and embedded in paraffin for routine histology. The other was snap-frozen in isopentane and stored in liquid nitrogen. For immunocytochemistry, 6-μm cryostat sections were cut, air-dried, and fixed in cold absolute acetone for 20 minutes. In all cases, a three-step, indirect immunoperoxidase procedure was performed. Serial sections were incubated for 30 minutes with the following monoclonal antibodies: mouse anti-human ICAM-1 (Becton-Dickinson, San Jose, CA), mouse anti-human VCAM-1 (Becton-Dickinson, San Jose, CA), mouse anti-human ELAM-1 (Genzyme Corp, Cambridge, MA), and mouse anti-human lymphocyte function-associated antigen-1 (Becton-Dickinson, San Jose, CA) at 1:50 working dilutions. Secondary staining was performed using avidin-biotin techniques. The sections were counter stained with 3-amino-9-ethylcarbazole (Biomeda Corp., Foster City, CA). Inflamed appendix was used as the control for antibodies ICAM-1, VCAM-1, and ELAM-1. Thymus was used as the control tissue for LFA-1 staining.

In control and study specimens, bile ducts were analyzed in three categories according to size. Proliferating ductules were defined as irregularly shaped biliary structures peripherally located and without discernible lumens. Interlobular bile ducts were defined as ducts with diameters ranging from 20 to 100 μ in diameter and lined by cuboidal ductal epithelium. Septal or trabecular ducts were defined as those greater than 100 μ in size. Additional structures analyzed included vascular endothelium (portal venous and arterial), sinusoidal lining cells including Kuppfer cells, hepatocytes, and infiltrating inflammatory cells. Extrahepatic ducts were analyzed in one case of biliary atresia and one of a choledochal cyst.

Results

Bile Ducts

None of the bile duct structures in any of the control specimens stained with ICAM-1 (Figure 1a). In marked contrast, sections from patients with biliary atresia showed intense staining of epithelial cells in all intrahepatic bile duct structures including proliferating bile ductules and interlobular ducts (Figure 1b). The single extrahepatic duct specimen also demonstrated strong ICAM-1 expression.

Of the other antibodies examined, only VCAM-1 occasionally stained bile duct/ductules in the biliary atresia cases. The staining was not as strong or as consistent as ICAM-1. In the control cases bile duct epithelium failed to stain with any of the other antibodies (VCAM-1, ELAM-1, OR LFA-1).

Hepatocytes/Sinusoidal Lining Cells

Hepatocyte membrane and sinusoidal lining cell staining with ICAM-1 and VCAM-1 was present in all specimens with inflammation. The intensity of staining with both antibodies correlated roughly with disease activity but was not related to underlying etiology. This staining pattern was found in all of the cases of biliary atresia (Figure 1c) and in the control cases of viral hepatitis, metastatic carcinoma, and TPN-induced liver disease. The liver specimen from the case with thrombotic thrombocytopenic purpura was devoid of inflammation and showed no staining with either antibody. ELAM-1 and LFA-1 did not show any hepatocyte membrane or sinusoidal lining cell staining.

Vascular Endothelium

As expected, ICAM-1 expression on vascular endothelial cells was found in all specimens. VCAM-1

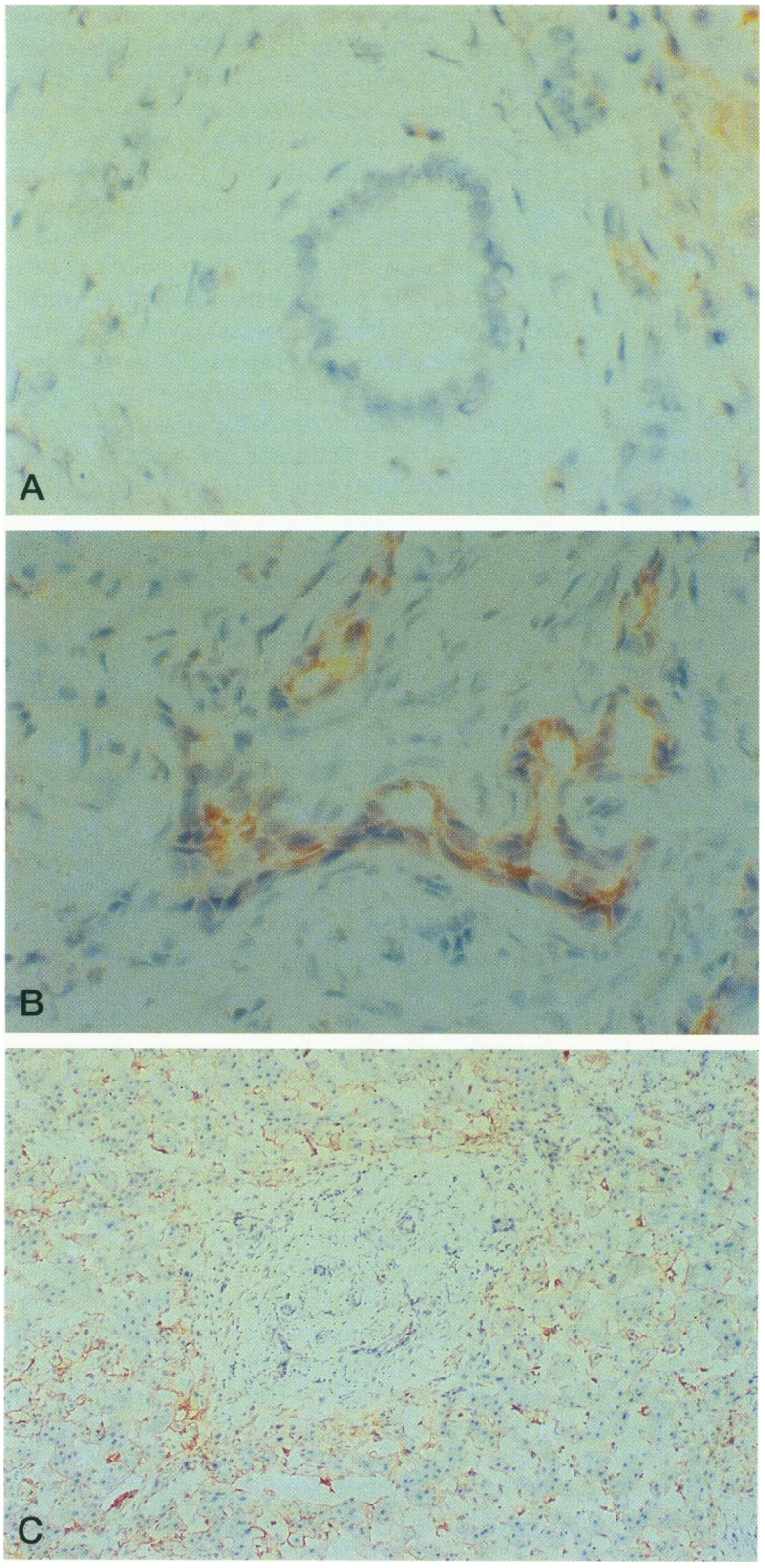

Figure 1. *Immunoperoxidase staining pattern of liver biopsies with anti-ICAM-1.* a: *Interlobular bile duct from a patient with viral hepatitis demonstrating lack of staining* (×400). b: *Bile ductule from a patient with extrahepatic biliary atresia demonstrating strong staining* (×400). c: *Hepatocyte membrane and sinusoidal lining cell staining in a case of biliary atresia. Note the positive bile ductules* (×200).

staining showed more variability than ICAM-1 in both intensity and consistency of endothelial cell staining. ELAM-1 and LFA-1 did not demonstrate any endothelial staining.

Inflammatory Cells

LFA-1 intensely stained the inflammatory cell infiltrate in the biliary atresia and inflamed control specimens. None of the other antibodies demonstrated binding to the inflammatory cells.

Discussion

The inflammatory response associated with biliary atresia involves both the extrahepatic and intrahepatic ductal systems. This chronic inflammation is associated with bile duct obliteration, bile stasis, and ductular proliferation as well as areas of hepatic cell injury resulting in peri-portal fibrosis and eventual cirrhosis. Although the exact etiology of this disease process remains unclear, it may be that immune-mediated mechanisms of damage to bile duct epithelium play a role in its pathogenesis. The strong expression of the adhesion molecule ICAM-1 on biliary ductal epithelium demonstrated by immunohistochemical analysis of liver biopsies from patients with biliary atresia suggests that the expression of this receptor may play a role in the pathogenesis of the disease process.

Recently, a number of inflammatory liver diseases has been associated with adhesion molecule expression. In cases of acute hepatitis ICAM-1 is strongly expressed throughout the liver parenchyma on both hepatocyte membranes and sinusoidal lining cells. A more focal pattern of expression is detected in cases of chronic active and chronic persistent hepatitis with ICAM-1 present only on hepatocytes in areas of periportal and intraacinar inflammation. In all areas of inflammation infiltrating lymphocytes are consistently positive for LFA-1 expression.[4,5] ELAM-1 is strongly expressed on sinusoidal lining cells throughout the liver parenchyma in acute hepatitis, whereas VCAM-1 expression is quite strong in areas of inflammation in chronic active and persistent hepatitis.[7] Thus, the strong expression of ICAM-1 in both acute and chronic liver inflammation and the differential expression of ELAM-1 in acute and VCAM-1 in chronic hepatic inflammation indicates a potential role for these adhesion molecules in the pathogenesis of hepatic disease.

Adhesion molecule expression has also been implicated in the pathogenesis of primary biliary cirrhosis and primary sclerosing cholangitis. Both conditions involve an inflammatory reaction of lymphocytes and mononuclear cells leading to the destruction of bile duct structures. In both conditions, strong expression of ICAM-1 has been found on the epithelial cells of interlobular bile ducts and proliferating bile ductules in patients with end-stage disease.[6] On the other hand, the absence of ICAM-1 expression on bile duct structures from patients with less advanced conditions of primary biliary cirrhosis and primary sclerosing cholangitis has recently been reported.[9] As a result, it has been proposed that the ductular expression of ICAM-1 in these processes correlated with the stage of the disease and degree of inflammation.

Our results show that neonatal biliary atresia has a pattern of adhesion molecule expression very similar to that seen in the end-stage processes of primary biliary cirrhosis and primary sclerosing cholangitis. In areas of inflammation, there is very strong expression of ICAM-1 on ductal epithelium and ICAM-1 and VCAM-1 on parenchymal and sinusoidal cells with no expression of ELAM-1. The accompanying mononuclear infiltrate is positive for LFA-1 expression. This immunohistochemical evidence for the strong expression of ICAM-1 on biliary epithelium and LFA-1 on infiltrating mononuclear cells is the first evidence that adhesion molecule pathways are involved in the pathogenesis of biliary atresia.

In contrast, bile ducts from the liver with total parenteral nutrition-induced cholestatic injury failed to express ICAM-1, and the liver specimen with hepatitis also failed to show ductal involvement. Similar results have been noted in adult liver specimens with cirrhosis and fulminant hepatitis, showing that bile duct proliferation itself does not result in vascular adhesion molecule expression. No other causes of cirrhosis, including cholestasis, have been found to induce ICAM-1 expression on bile duct structures.[8] It seems that in those diseases where bile duct destruction is central to the pathological process, ICAM-1 expression is a unique finding. Thus, a mechanism can be proposed for the pathogenesis of biliary atresia involving a primary inflammatory ductal insult marked by the up-regulation of adhesion molecule expression on bile duct epithelium and the subsequent recruitment of lymphocytes and monocytes resulting in biliary tract destruction and parenchymal damage.

We believe that this work may have exciting therapeutic implications for biliary atresia, because currently there is no therapy except for surgical palliation with a Kasai hepatoportoenterostomy or hepatic transplantation. We speculate that the pharmacological blockade of expressed ICAM-1 or perhaps the prevention of its cell-surface expression could con-

ceivably minimize or eliminate the resulting bile duct damage. Little is known of the effect of immunosuppressive agents on intercellular adhesion molecule expression in biliary epithelium, though steroid administration has reduced ICAM-1 expression in rejecting liver transplants.[10] Monoclonal antibody therapy against ICAM-1 has prolonged the survival of renal allografts in nonhuman primates, and such therapy is currently undergoing clinical evaluation in renal transplantation, burn wound treatment, and rheumatoid arthritis protocols.[11] Such therapies may provide new possibilities in treating this devastating neonatal disorder.

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