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Differential Expression of the Major Histocompatibility Antigens and ICAM-1 on Bile Duct Epithelial Cells in Biliary Atresia

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DILLON, P.W., BELCHIS, D., MINNICK, K. and TRACY, T. *Differential Expression of the Major Histocompatibility Antigens and ICAM-1 on Bile Duct Epithelial Cells in Biliary Atresia.* Tohoku J. Exp. Med., 1997, **181** (1), 33-40 —— Aberrant expression on biliary epithelial cells of the major histocompatibility complex (MHC) antigens in association with adhesion molecule intercellular adhesion molecule-1 (ICAM-1) may be crucial to the immunopathogenesis of biliary atresia. The patterns of MHC class I and II expression in relation to ICAM-1 expression as well as the associated lymphocyte subpopulations were studied in frozen section liver biopsies from six infants with biliary atresia. Intense ICAM-1 expression was found on all ductal epithelial cells in association with MHC I. No ductal epithelial cells demonstrated MHC II expression. Lymphocyte populations within the portal tracts all expressed LFA-1 and were predominantly CD4 positive ($>70\%$). CD8 positive cells accounted for less than 30%. The expression of ICAM-1 appears to be important in the pathogenesis of biliary atresia but is not linked to the expression of MHC II determinants. This result suggests that different regulatory mechanisms govern the expression of these important immunological receptors on biliary epithelial cells. ———— biliary atresia; major histocompatibility antigens (MHC); intercellular adhesion molecule-1 (ICAM-1); CD4; CD8

The inflammatory response associated with biliary atresia involves both the extrahepatic and intrahepatic ductal systems. This chronic inflammation results in bile duct destruction and obliteration, bile stasis, and ductular proliferation.

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The infiltration of lymphocytes and macrophages into the portal tracts suggests that immunological pathways are involved in this destructive process with the ductal epithelial cell as the target. However, the immunologic mechanisms controlling this process are unknown.

The recruitment of lymphocytes and other immune effector cells into a tissue site is regulated by the expression of various adhesion molecules of which intercellular adhesion molecule-1 (ICAM-1) is prominent (Springer 1990). These molecules govern the adhesion and migration of leukocytes into target tissues as the first steps of an immunological reaction. The overall immune response and the ability to process and recognize antigenic determinants is governed by the major histocompatibility class of antigens (Benacerraf 1981). Studies have shown that in organ specific autoimmune states aberrant expression of MHC determinants by epithelial cells may play a role in the pathogenesis of these diseases (Hanafusa et al. 1983).

Biliary epithelial cell expression of both ICAM-1 and MHC cell surface receptors has been implicated in the ductal destruction of the adult diseases of primary sclerosing cholangitis (PSC) and primary biliary cirrhosis (PBC) (Van den Oord et al. 1986; Chapman et al. 1988; Broome et al. 1990, 1993; Adams et al. 1991). In biliary atresia ICAM-1 expression has also been demonstrated on ductal epithelial cell membranes in a pattern similar to PSC and PBC (Dillon et al. 1994). We hypothesized that aberrant ICAM-1 and MHC expression occurred on ductal epithelial cells in infants with biliary atresia as part of the immunopathology of the disease process. Therefore, we examined the pattern of expression of MHC class I and II antigens in relation to ICAM-1 expression on bile duct structures of patients with this devastating neonatal hepatobiliary disorder.

Materials and Methods

Patients

Liver samples from six infants were analyzed. These specimens were obtained from infants ranging in age from 4 to 10 weeks undergoing surgical biopsies for the evaluation of hyperbilirubinemia. All had biliary atresia. The diagnosis was 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 formalin fixed and embedded in paraffin for routine histology. The other was snap-frozen in isopentane and stored in liquied nitrogen. Cryostat cut sections were incubated with antibodies against CD4 (mouse anti-human inducer T-cell; 1 : 40; T4[SFCI 12 T4T11] Biogenex, San Ramon, CA, USA), CD8 (mouse anti-human suppressor/cytotoxic T cells; T8[SFCI 21 Thy], Biogenex, San Ramon), and HLA-DR (mouse anti-human HLA-DR, L243, Becton Dickinson,

San Jose, CA, USA). CD4 and CD8 were analyzed using a three step procedure by the avidin-biotin peroxidase complex technique (Falini and Taylor 1983). Tissue sections were incubated with the primary antibody at room temperature for 4 hr.

A biotinylated antibody to HLA-DR was analyzed using a two step avidin-biotin complex technique. Tissue sections were incubated with the primary antibody for 1 hr at 37°C. ICAM-1 and LFA-1 studies were performed as described previously (Dillon et al. 994). Formalin fixed, paraffin embedded specimens were stained for β_2-microglobulin-a marker for MHC I.

Positive controls performed concurrently consisted of thymus for CD4 and CD8 and tonsil for HLA-DR.

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 wih diameters ranging from 20 to 100 μm in diameter and lined by cuboidal ductal epithelium. Septal or trabecular ducts were defined as those greater than 100 μm in size. Additional structures analyzed included vascular endothelium (portal venous and arterial), sinusoidal lining cells including Kuppfer cells, hepatocytes, and infiltrating inflammatory cells.

Results

Bile ducts

As previously reported, ICAM-1 expression was not detected on the bile duct epithelial cells in control specimens (Dillon et al. 1994). In patients with biliary atresia intense ICAM-1 staining was detected on epithelial cells in all intrahepatic bile duct structures including proliferating bile ductules and interlobular ducts (Fig. 1). β_2-Microglobulin (MHC I) expression of variable intensity was detected on the epithelial cell membranes of all bile ducts and ductules in both control and atresia specimens (data not shown). The staining pattern was not uniform and varied between bile duct structures within the same portal tract. No expression of the MHC II antigen (HLA-DR) was detected on the ductal epithelial cells in either the control or atresia specimens (Fig. 2). Occasional peri-ductal spindle cells were positive for HLA-DR expression.

Sinusoidal lining cells/Kuppfer cells

Hepatocyte membrane and sinusoidal lining cells staining with ICAM-1 was present in all specimens with inflammation. β_2-Microglobulin staining was found on sinusoidal lining cells, Kuppfer cells, and fibroblasts in the portal tract. HLA-DR was strongly expressed on Kuppfer cells, sinusoidal lining cells in a patchy distribution, and fibroconnective tissue. Sinusoidal lining cells were positive for CD4 expression but negative for CD8 (Fig. 3). Kuppfer cells strongly expressed CD4 but showed variable and patchy expression of the CD8 determi-

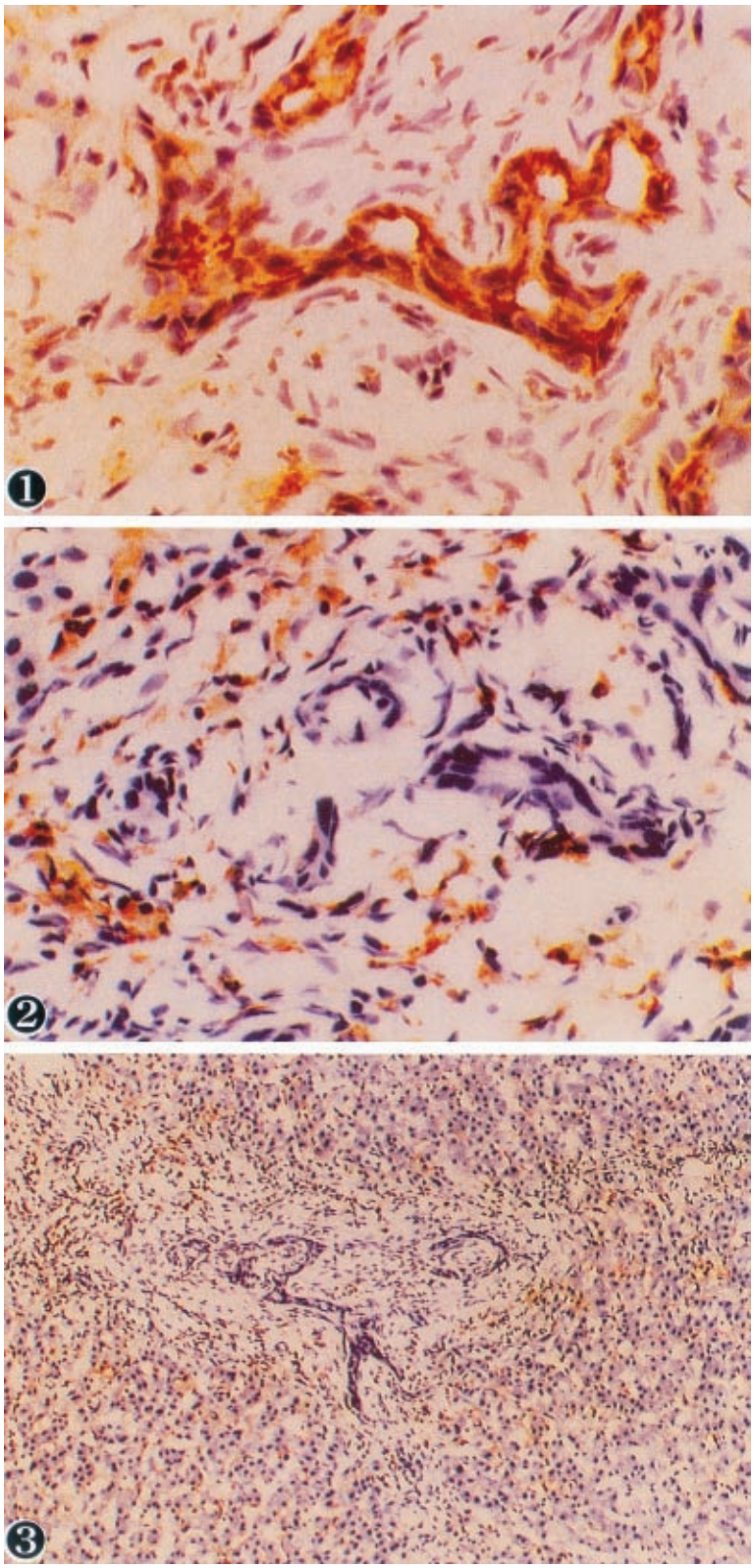

Fig. 1. ICAM-1: Strong expression by biliary ductule epithelial cells in a case of biliary atresia.

Fig. 2. HLA-DR: Lack of expression by luminal biliary epithelial cell. Strong expression by inflammatory cells.

Fig. 3. CD4: Strong expression on inflammatory cells, Kuppfer cells, and sinusoidal lining cells.

nant.

Vascular endothelium

As expected, ICAM-1 expression on vascular endothelial cells was detected in all specimens. β_2-Microglobulin was also detected diffusely on the vascular endothelium. Vascular endothelium did not express either CD4 or CD8.

Inflammatory cells

LFA-1 intensely stained the inflammatory cell infiltrate in all specimens as did β_2-microglobulin and HLA-DR. Greater than 70% of the portal lymphocytes were positive for CD4 expression (Fig. 3) in contrast to 30% or less of the portal lymphocytes demonstrating CD8 expression (data not shown). Many of the portal inflammatory cells were positive for β_2-microglobulin. Sinusoidal lining cells also expressed CD4.

Discussion

Though the etiology of biliary atresia remains unknown, this study demonstrates that altered immunologic mechanisms may be involved in its pathogenesis. The basis for any immune response involves the recruitment of lymphocytes into a target tissue aided by adhesion molecule expression. This process of migration is then followed by the processing of antigenic signals by MHC class I or II determinants to differentiate and activate the recruited cells. Knowing that ICAM-1 expression is involved in a number of immune functions including lymphocyte adhesion and cell-mediated cytotoxicity, its strong expression on ductal epithelium in biliary atresia suggests that it may play a role in the progressive damage to bile duct structures. Ductal expression of MHC II determinants does not appear to influence this process, since none of the biliary epithelial cells and only a few peri-ductular cells were positive for HLA-DR expression. On the other hand, MHC I expression was demonstrated throughout ductal and parenchymal structures and thus may serve as the major immunological determinant.

Abnormal ICAM-1 and MHC expression have been demonstrated in the pathogenesis of the ductal destructive processes of primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) in adults (Van den Oord et al. 1986; Chapman et al. 1988; Broome et al. 1990, 1993; Adams et al. 1991). Both conditions involve an inflammatory reaction in the portal tract leading to the destruction of ductal structures. In both conditions, strong expression of ICAM-1 has been found on the epithelial cells of interlobular bile ducts and proliferating bile ductules with end-stage disease (Adams et al. 1991). Altered MHC expression has also been demonstrated in both conditions with particular emphasis on increased ductal HLA-DR expression. Such aberrant expression has been proposed as evidence for an autoimmune basis for these disease processes (Chapman et al.

1988). However, conflicting results have been obtained when the simulataneous expression of both markers has been examined in these diseases. Broome et al. (1993) found that the co-expression of ICAM-1 and HLA-DR on involved bile ducts did not occur in PBC, while Bloom et al. (1995) and Yasoshima et al. (1995) found a high degree of concomitant ductal epithelial expression in both pathologic conditions. ICAM-1 expression was also somewhat variable in these studies leading to the proposal that its expresssion in PSC and PBC did not play a role until late in the disease process. In biliary atresia our results have been different in regards to ICAM-1 with its uniform expression in all postnatal specimens (Dillon et al. 1994). The results in this study have shown that it is MHC expression that may be variable as part of the immunopathogenesis of the disease.

Increased expression of MHC II determinants has been reported in patients with biliary atresia by Muraji et al. (1988). They found that 4 of 5 frozen section liver biopsies and 11 of 15 formalin fixed specimens demonstrated HLA-DR expression. In the formalin fixed specimens the negative results reportedly came from livers biopsied late in the neonatal course as part of reoperative surgery and imply a time factor governing such expression. The contradictory results between their study and ours may result from differences in the timing of the surgical biopsies or in the immunostaining techniques and antibodies used. As in PSC and PBC, MHC expression in biliary atresia may be temporally related to the stage and extent of the disease.

The lack of concomitant expression of ICAM-1 and HLA-DR on the bile ducts in biliary atresia indicates that different regulatory mechanisms or pathways of stimulation may exist for the cholangiocyte. The expression of both cell surface receptors can be induced by the inflammatory mediators IFN-g and IFN-a (Griffith et al. 1989). As such, concomitant expression of both markers would be expected under classical inflammatory pathways. These results in conjunction with those of Broome et al. (1993) indicate that biliary epithelial cells may respond differently to inflammatory mediators than vascular endothelium or mesenchymal cells due to any number of reasons. For example, as a result of interactions with surrounding hepatocytes, there may be modifying factors in the hepatic microenvironment altering cholangiocyte inflammatory responses. Bile acids have an inhibitory effect on TNF-a release from mononuclear cells, and the administration of such acids has altered MHC I expression in PBC (Greve et al. 1989; Calmus et al. 1990). Whether bile acids have any effects on the inflammatory response of biliary epithelial cells remains to be evaluated.

Though the etiology of biliary atresia remains unknown, there is increasing evidence that the disease could be an autoimmune disorder (Vasiliauskas et al. 1995). MHC genotype has been linked to a number of disease processes and has involved both class I and II molecules. Class I MHC hyperexpression has been demonstrated on pancreatic islet cells of diabetic patients and may render such cells more susceptible to lysis by cytotoxic lymphocytes as part of the autoimmune

response. Experimentally, TNF-a and IFN-g have been found to enhance this expression on islet cells in both humans and animals (Campbell et al. 1988). If such a response exists for biliary epithelial cells, then the concomitant expression of ICAM-1 and MHC I could be linked through cytokine pathways as a potential immunologic mechanism for the disease. Bile duct destruction would then occur by classic cytotoxic mechanisms mediated by CD8 positive lymphocytes with epithelial-lymphocyte adhesive interactions strengthened by the associated ICAM-1 upregulation.

On the other hand, it is interesting that this work as well as others have shown a higher percentage of $CD4^+$ T cells in the portal tract than $CD8^+$ cells in biliary atresia (Chen et al. 1989; Endo et al. 1989). Similar findings have been reported for PSC and PBC (Si et al. 1984). This predominance of $CD4^+$ lymphocytes in the portal tract region raises the possibility of an alternative mechanism of immune mediated ductal injury and epithelial cell death. It is now well documented that $CD4^+$ T cells can exhibit cytotoxic activity and that such a process exists to serve as a regulatory force against activated MHC II positive cells (Hahn et al. 1995). Although the activation of $CD4^+$ cytotoxic lymphocytes is MHC class II restricted, the cytotoxic or effector phase is unrestricted and antigen non-specific. This non-specific killing is mediated by Fas-FasL interactions and has been implicated in bystander cell lysis responsible for certain autoimmune diseases and in virus induced hepatitis C (Hiramatsu et al. 1994). The extent of the destruction and hence the virulence of the disease process would potentially be governed by the degree or time course of MHC II expression. The possiblity of such a mechanism at work in biliary atresia remains to be evaluated.

This study raises a number of interesting possibilities for potential immunlogic mechanisms involved in the pathogenesis of biliary atresia. Further understanding of these mechanisms may ultimately lead to attempts to modulate the inflammatory reaction that appears to target the biliary epithelial cell for destruction and provide for improved methods of treating this devstating neonatal hepatobiliary disorder.

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