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Increased CXCR3 expression associated with CD3-positive lymphocytes in the liver and biliary remnant in biliary atresia

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Abstract

Background: Lymphocyte-mediated inflammatory damage of the bile ducts has been proposed as a potential mechanism in the pathogenesis of biliary atresia (BA). Chemokines regulate leukocyte migration and act as critical organizers of cell distribution in inflammatory responses. The aim of this study was to analyze the infiltration of T lymphocytes and the expression of a chemokine receptor, CXCR3, predominantly expressed on type 1 polarized T cells (T_H1, T_C1) in the liver and excised biliary remnants in infants with BA.

Methods: Immunohistochemistry for CD3, CD8, and CXCR3 was performed using liver biopsy specimens collected from the following 3 age-matched groups of patients: group 1, BA (non-syndromic) at the time of Kasai portoenterostomy (n = 10); group 2, congenital choledochal dilatation (n = 2); and group 3, other cholestatic diseases including paucity of intrahepatic bile ducts and cholestasis (n = 3) related to total parenteral nutrition. Cellular staining on each section was graded from 0 to 4 and compared using nonparametric statistics.

Results: Infiltrating $CD3^+$ and $CD8^+$ lymphocytes in the portal tracts were significantly increased in group 1 (3.1 ± 0.4, 2.8 ± 0.4), compared with groups 2 (1.0 ± 0.0, 1.0 ± 0.0) and 3 (1.7 ± 0.3, 1.5 ± 0.5) ($P < .01$, $P < .05$). $CXCR3^+$ mononuclear cells were significantly increased in group 1 (2.6 ± 0.3) compared with groups 2 (0.5 ± 0.5) and 3 (0.7 ± 0.3) ($P < .05$). They were mainly found in the portal tracts with a similar distribution to $CD3^+$ cells. $CXCR3^+$ cells and $CD3^+$ cells also showed a similar distribution in specimens of biliary remnants from just below the portal plate.

Conclusions: Increased expression of CXCR3 associated with a significantly increased CD3 and CD8 T-cell infiltration suggests that $CXCR3^+$ lymphocytes in a type 1 (T_H1, T_C1) cytokine milieu may play a role in the pathogenesis of BA.

Biliary atresia (BA) is an inflammatory fibrosing condition affecting the extrahepatic and intrahepatic biliary tree, resulting in fibrous obliteration of the biliary tract and the development of cirrhosis [1,2]. Biliary atresia is generally classified into 2 distinct phenotypes, syndromic and nonsyndromic, according to the presence of extrahepatic

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Table 1 Patient characteristics

Patient group (n)	Disorder	Sex (male-female)	Mean age, d (range)
1 (10)	BA (nonsyndromic)	3:7	57 (28-90)
2 (2)	Choledochal cyst	1:1	58 (57-58)
3 (3)	Biliary hypoplasia	2:1	71 (67-111)
	Idiopathic neonatal hepatitis		
	PN-associated cholestasis		

PN indicates parenteral nutrition.

anomalies. Although there are numerous hypotheses about its pathogenesis, the cause of BA is still unknown [1,3]. Whatever the cause, common histologic findings in the extrahepatic biliary remnant include periductal inflammation with loss of the epithelial lining and progressive periductal fibrosis and obliteration of the biliary lumen [4]. Intrahepatic portal tracts are also involved in this inflammatory progression [5]. Various studies of the mechanisms of inflammation in BA have implicated Kupffer cells, T lymphocytes, and natural killer (NK) cells in an immunologically mediated process of bile duct obstruction [6-8].

Among the various mechanisms of leukocyte recruitment (complement components, leukotrienes, platelet activating factor, etc), most dynamic roles are played by chemotactic cytokines, termed *chemokines*. The chemokines are a superfamily of small proteins composed of distinct amino acid sequences, and most of the members have a specialized capacity to directly and differentially chemoattract specific subsets of leukocytes [9]. They are subdivided based on the number and positioning of their highly conserved cysteines (CC, CXC, CX3C, C), and the receptors for chemokines are 7 transmembrane-spanning, G protein–coupled receptors. The chemokine receptor, CXCR3, has been demonstrated predominantly on T lymphocytes, including activated $CD4^+$ T lymphocytes of the T_H1, $CD8^+$ cytotoxic T cells of type 1 phenotype (T_C1), and NK cells [10]. The receptor binds 3 ligands, CXCL9/monokine induced by interferon γ (IFN-γ) (Mig), CXCL10/IFN-γ–inducible protein 10, and CXCL10/interferon-inducible T cell-α chemoattractant. CXCR3 and its ligands have been reported to play a pivotal role during inflammatory bowel disease [11], inflammatory skin disease [12], chronic viral hepatitis [13], and allograft rejection [14].

The aim of this study was to analyze the infiltration of T lymphocytes and the expression of a chemokine receptor CXCR3 in the liver and excised biliary remnants in infants with BA.

1. Materials and methods

1.1. Patients and specimens

Wedge liver biopsies (segment 4) from 15 infants ranging between 28 and 111 days of age were studied. Ten biopsies were obtained from infants with BA at the time of Kasai portoenterostomy (group 1). In each case, type 3 BA (atresia at the porta hepatis) was confirmed at operation and by histology of the excised biliary remnants. None of these patients had features of the BA splenic malformation syndrome. Two liver biopsies were obtained from 2 infants with congenital choledochal dilatation at the time of choledochal excision (group 2). Liver biopsies were also obtained from 3 infants, with other cholestatic disorders including biliary hypoplasia (n = 1), idiopathic neonatal hepatitis (n = 1), and parenteral nutrition–associated cholestasis (n = 1), who required an operative cholangiogram to exclude BA (group 3). The demographic data of these patients are shown in Table 1.

1.2. Immunohistochemistry

All wedge liver biopsy specimens were fixed in neutral 10% formalin for 24 to 48 hours at room temperature and embedded in paraffin. Sections of 4 μm thickness were cut and placed on Superfrost slides (VWR International, Lutterworth, Leicestershire, UK). After deparaffinization, endogenous peroxidase activity was quenched with 0.3% (vol/vol) hydrogen peroxide in methanol for 30 minutes at room temperature (*T*). Antigen retrieval was performed by boiling the sections in 0.01 mol/L citrate buffer (pH 6.0) for 10 minutes in a microwave at 800 W. Nonspecific binding sites were blocked using 10% (vol/vol) normal goat serum with or without 1% (wt/vol) bovine serum albumin and 10% (vol/vol) fetal calf serum for 30 minutes at *T*. Tissue sections were incubated with primary antibodies overnight at 4°C. After washing in Tris-buffered saline, the sections were incubated for 30 minutes at *T* with goat antimouse immunoglobulins conjugated to a peroxidase-labeled polymer (Envision kit; DakoCytomation, Glostrup, Denmark). Immunoreacted cells were then visualized with 3,3′-diaminobenzidine solution. The sections were counterstained with hematoxylin. Primary antibodies, mouse monoclonal antibodies against human CD3 (PS1), CD8 (1A5), and CD57 (NK1) were purchased from Novocastra Laboratories (Newcastle upon Tyne, UK). Mouse monoclonal antihuman CXCR3 (1C6) was purchased from BD Bioscience (San Diego, Calif).

1.3. Histologic Scoring

Semiquantitative analysis of infiltrating positive cells in the portal tract was performed for each antibody. The portal tracts in each specimen were photographed at magnification ×200 with a Leica DC digital camera (Leica Microsystems, Glattbrugg, Switzerland) attached to a light microscope (Leica DML). Immunostained sections were evaluated independently in blinded fashion without knowing the diagnosis of the patients. The semiquantitative method reported previously [7] was used to grade infiltrating cells as follows: 0, absent; 1, minimal/occasional cells; 2, 1 focal infiltrate;