Experimental Obliterative Cholangitis

A Model for the Study of Biliary Atresia

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Noninfectious obliterative cholangitis results from biliary tract inflammation in clinical conditions such as biliary atresia and sclerosing cholangitis. The purpose of this study was to develop an animal model of noninfectious biliary tract inflammation and fibrosis. An implantable osmotic pump was connected to a catheter placed into the gallbladder of hamsters. Phorbol myristate acetate (PMA) was infused into the biliary tract for periods of 6 hours to 28 days. After 7 days the animals developed neutrophil infiltration, cellular necrosis, and edema of the biliary ducts. After 14 days, the animals demonstrated intrahepatic cholestasis with bile duct fibrosis and acute and chronic inflammatory cell infiltration. By 28 days pronounced portal fibrosis was present, some of which created an early bridging cirrhosis pattern. In addition there was evidence of neocholangiogenesis. We conclude that long-term PMA infusion into the biliary tract generates an inflammatory response characterized by obliterative cholangitis and fibrosis, sharing many of the histologic features of human biliary atresia. This model may provide a relatively simple technique for investigating the process of nonpyogenic biliary tract inflammation.

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BILIARY ATRESIA WAS formerly considered an accident in embryogenesis which resulted in nonpatency of the extrahepatic biliary tree. However, more recent evidence suggests that this is not the case.[1] Clinical evidence of obstructive jaundice usually is not present at birth but typically appears at several weeks of age. In addition Kasai et al.[2,3] provided clinical and histopathologic evidence that patent bile ducts are present in the first 2 months of life but gradually disappear, so that after 4 months of age only fibrous, nonpatent ducts remain. Further evidence against an embryonic pathogenesis for biliary atresia is provided by the observation that it is ordinarily not associated with congenital anomalies of other major systems, one of the characteristic features of discrete teratogenic events.[4] Last biliary atresia is an exceedingly uncommon finding at autopsy of aborted fetuses or neonates, suggesting a postnatal disease process.[5]

A variety of other explanations for this condition have been offered, including malunion of the pancreatic and biliary ductal systems,[6] fetal vascular accidents,[7-9] and perhaps most convincingly, viral infections.[10-12] In 1977 Bill et al.[5] pointed out the prominent inflammatory response associated with biliary atresia and proposed that the disease might result from a dynamic, acquired inflammatory process. Subsequently other investigators implicated the Reovirus type III as a potential pathogen. Experimental studies in mice and epidemiologic data from human studies provided support for this concept.[10-12] The concept also was developed that biliary atresia results not necessarily from a specific pathogen but rather from a nonspecific activation of the endogenous inflammatory system. The mechanisms by which the liver and biliary tree of the neonate become targeted, and the specific pathogenic mediators involved, are unknown.

The purpose of this investigation was to evaluate the hypothesis that the initiation of acute inflammation in the biliary tract with a nonspecific trigger such as phorbol myristate acetate (PMA) would result in chronic inflammation, fibrosis, and obliteration of the bile ducts. These histologic changes are characteristic of chronic nonpyogenic inflammation of the biliary ducts in humans; in children the common process with these characteristics is biliary atresia; in adults sclerosing cholangitis is the notable example. The study suggests that inflammation and fibrosis involving the bile ducts can be induced with PMA and that the histologic features of this injury resemble those seen in humans with biliary atresia and other in-

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flammatory biliary processes. In addition the known relationship of PMA as a potent stimulator of oxygen radical production by stimulated phagocytic cells suggests that there may be a relationship between this type of biliary inflammation and oxygen radical-mediated tissue injury.

Materials and Methods

Implantable mini-osmotic pumps (Alza Corp., Palo Alto, CA) were prepared with either normal saline or PMA in saline (Sigma Chemical Co., St. Louis, MO). Pumps varied in size and capacity to deliver the infusate at a constant rate (model 2001-250 μL at 0.46 μL/hr for 7 days; model 2002-250 μL at 1.03 μL/hr for 14 days; model 2ML-2200 μL at 2.69 μL/hr for 28 days) during a predetermined time period for 7 to 28 days. Animals in the 6-hour study group were given a single injection of PMA or saline. All infusions were through a polyethylene catheter (PE 60) placed surgically into the gallbladder. The dose of PMA in all study groups was 25 μg/kg/day. In each case the pump and tubing were primed before implantation.

Experimental protocols were approved by the University of Michigan Committee on Use and Care of Animals. Male Syrian golden hamsters weighing 80 g (Charles River Laboratories, Wilmington, MA) were anesthetized with ketamine hydrochloride (50 mg/kg intramuscularly). Using aseptic techniques the liver and gallbladder were exposed through a right subcostal incision. A 6-0 silk purse string was placed in the fundus of the gallbladder. A subcutaneous pocket then was created in the right flank, the pump inserted, and the polyethylene tubing tunneled through the abdominal wall. The polyethylene tubing then was placed into the fundus of the gallbladder and secured. The abdomen was irrigated with sterile saline, closed, and the animals were returned to standardized housing with rodent chow and water available *ad libitum*.

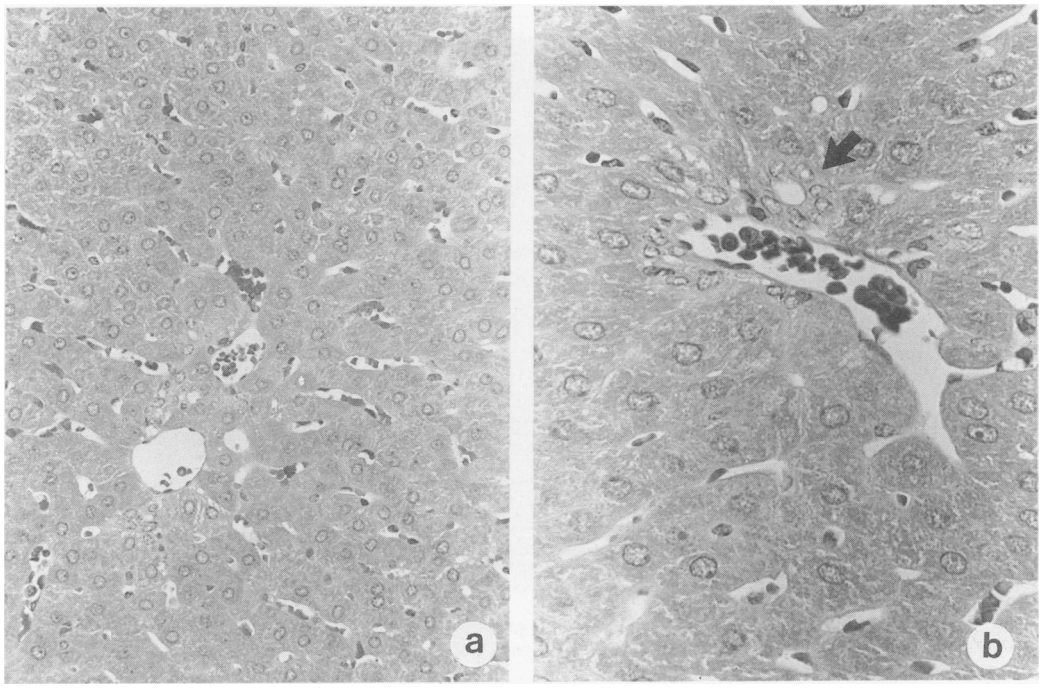
Animals were divided randomly into groups of five each according to the time of PMA or saline infusion. Study groups were prepared with infusions lasting 6 hours, 7, 14, or 28 days with PMA or saline.

At the designated time that the animals were killed, the animals were anesthetized with ketamine as before and their subcostal incisions were re-entered. The liver, gallbladder, and pancreas were resected *en bloc* and preserved in formaldehyde. Routine processing for histologic examination was accomplished and tissues were stained with hematoxylin and eosin as well as trichrome elastic stains. Slides were examined randomly by a pathologist blinded as to treatment.

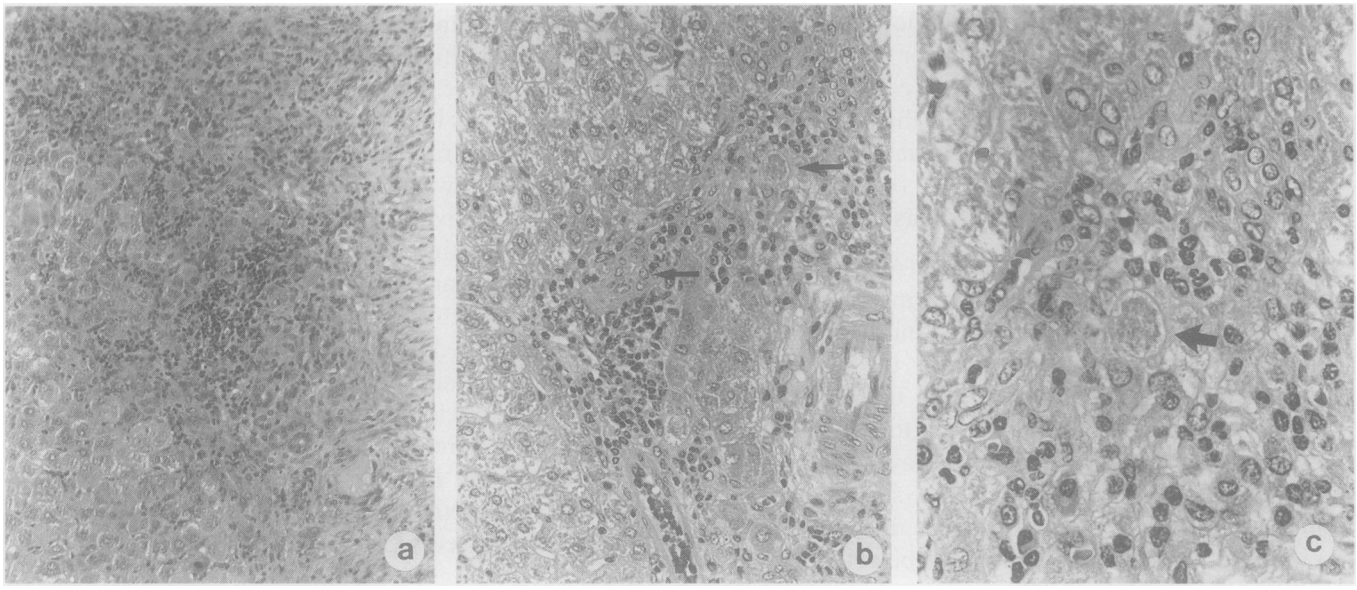
Results

Saline-infused animals demonstrated normal hepatic and biliary architecture at all times. Figure 1 illustrates the liver of an animal infused for 28 days with saline. There is no evidence of periportal inflammation or fibrosis.

In contrast to the animals infused with saline, those infused with PMA developed striking periductal inflammatory alterations. A single injection of PMA resulted in significant acute periductal inflammation in the liver with



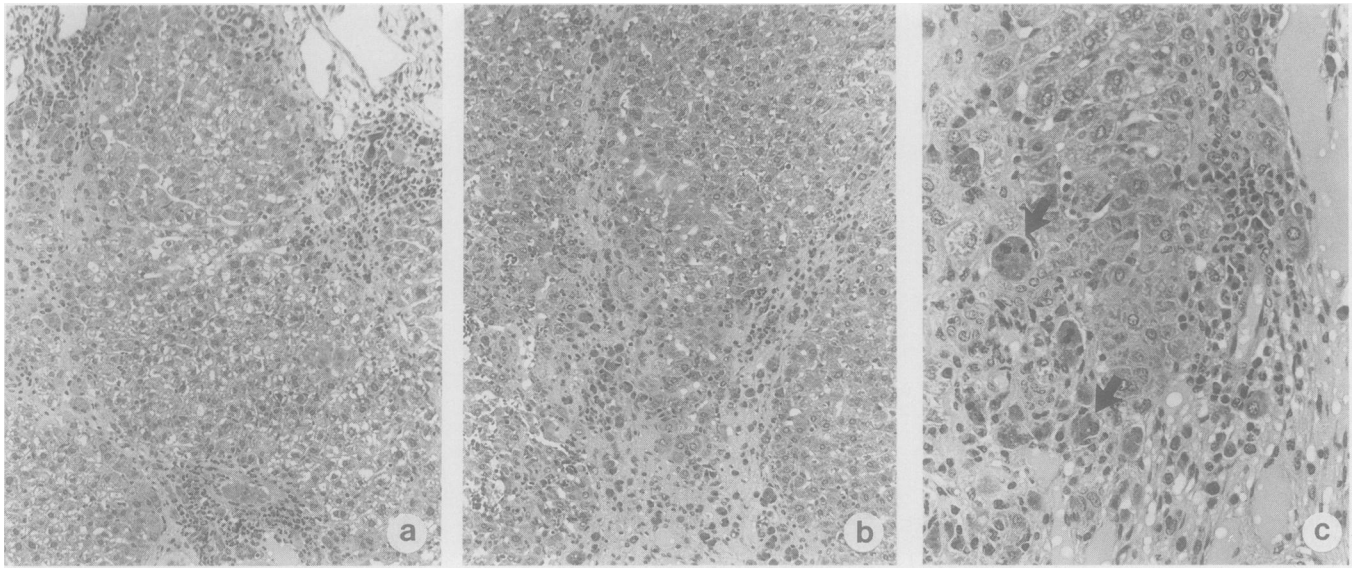
FIGS. 1a and b. Histologic appearance of the liver of a control animal given a saline infusion for 28 days. (a) A low-power view of a hepatic lobule illustrates no abnormality. (b) A high-power view of a normal portal triad is shown, including a bile duct as pointed out by the arrow (hematoxylin and eosin stain; a, 200×; b, 400×).



FIGS. 2a–c. Histologic alterations in the liver of animals given PMA infusion for 7 days. There is an intense periportal inflammatory infiltrate with extension into surrounding hepacytes, as illustrated in a. This inflammatory infiltrate was associated with destruction of bile ducts, as illustrated in b (arrows). Cholestasis was also present with a bile plug in a destroyed bile duct illustrated in c (arrow) (hematoxylin and eosin stain; a, 100×; b, 200×; and c, 400×).

neutrophilic infiltrates, edema, and some patchy necrosis of the bile ducts. After 7 days of PMA infusion, more severe inflammatory changes were present. As illustrated in Figure 2, pronounced inflammatory infiltrates were present in the livers of these animals. These infiltrates were primarily periductal but also extended into the surrounding liver parenchyma. At this time the inflammatory infiltrate was primarily acute in nature with a predominance of neutrophils, but chronic inflammatory cells such as macrophages, lymphocytes, and plasma cells were also present. The inflammatory infiltrate was associated with destruction of the bile ducts and focal hepatocyte necrosis was also evident. Prominent cholestasis was present within hepatocytes as well as in the bile ducts.

In animals infused with PMA for 14 days, a progression of the inflammatory response was seen with early fibrosis as well on ongoing inflammation and necrosis. This is illustrated in Figure 3. At this time a mixed acute and



FIGS. 3a–c. Histologic alterations in the liver of animals given PMA infusions for 14 days. There was ongoing intense periportal inflammation with the destruction of bile ducts, as illustrated in a. Early fibrosis was present in the portal regions (Figure 3B) and prominent cholestasis was present (c, arrows) (hematoxylin and eosin stain; a, 100×; b, 200×; and c, 400×).

chronic inflammatory infiltrate was present with a majority of the inflammatory cells consisting of lymphocytes, macrophages, and plasma cells. Despite the predominant chronic inflammatory infiltrate, ongoing tissue injury was present with destruction of the bile ducts and patchy hepatocyte necrosis. Prominent cholestasis was present in some of the hepatocytes and bile ducts. Early fibrosis was present, including some delicate bridging scarring of the liver parenchyma, indicative of early cirrhosis.

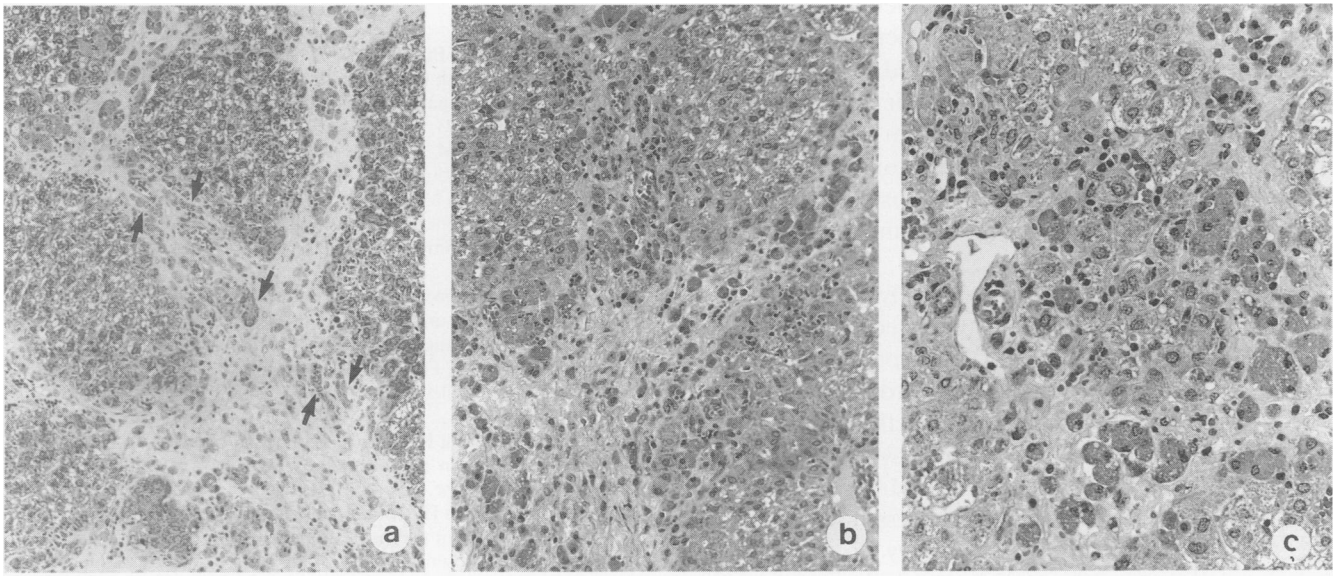
After 28 days of PMA infusion, the primary histologic appearance of the liver was that of scarring and early cirrhosis. As shown in Figure 4, bands of collagen separate lobules of hepatocytes creating the characteristic appearance of micronodular cirrhosis. Some inflammation per- that it is ordinarily not associated with congenital anomalies of other major systems, one of the characteristic features of discrete teratogenic events.[4] Last biliary atresia is an exceedingly uncommon finding at autopsy of aborted fetuses or neonates, suggesting a postnatal disease process.[5]

The above histologic alterations seen with PMA infusion are similar to those described in patients with noninfectious cholangitis secondary to biliary atresia or sclerosing cholangitis. This is illustrated in Figure 5, which represents varying stages of the histologic abnormalities present in liver biopsies from patients with biliary atresia. Figure 5A shows prominent periductal inflammation with destruction of bile ducts that occurs primarily early in the course of the disease. Figure 5B shows a liver biopsy from a child with long-standing jaundice secondary to biliary atresia. Prominent periportal fibrosis is present along with disordered bile duct epithelial proliferation (neocholangiogenesis). Some chronic inflammatory cells are present, but the primary histologic diagnosis is that of early micronodular cirrhosis. Thus the characteristic morphologic alterations seen in patients with biliary atresia are reproduced in this experimental model.

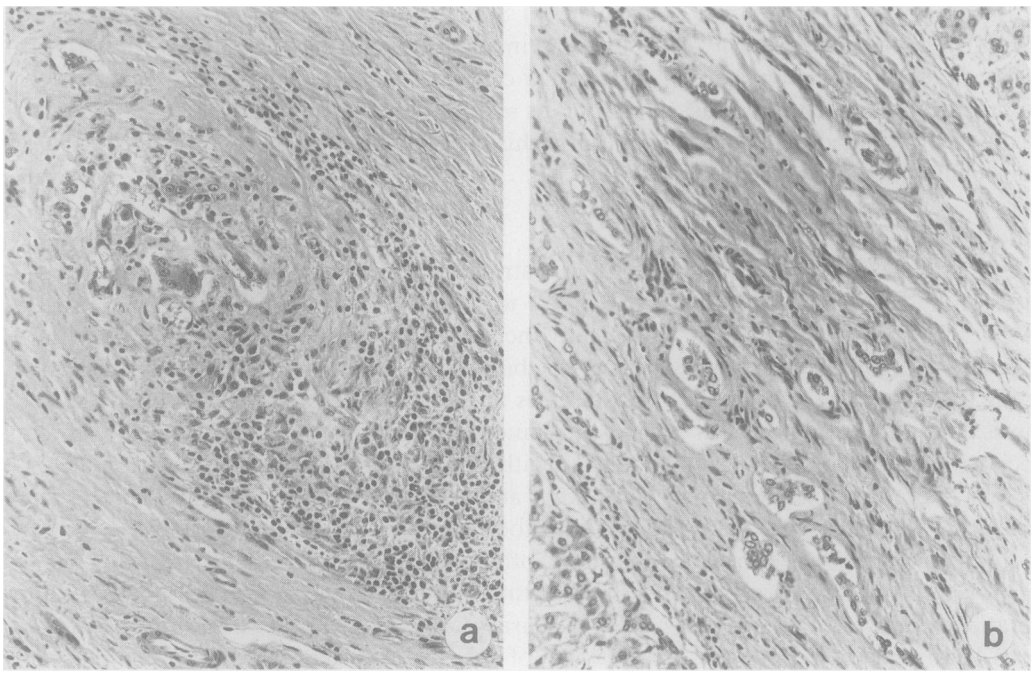
Discussion

Biliary atresia occurs with an estimated frequency of 1 in 15,000 to 25,000 neonates.[13,14] The histopathology typically includes extrahepatic bile duct fibrosis with intrahepatic bile duct proliferation. Inflammatory cells (both neutrophils and macrophages) are characteristic in both locations and ultimately cirrhosis may develop. Patients afflicted with biliary atresia continue to suffer predictable morbidity and mortality despite surgical advances such as the portoenterostomy procedures and liver transplantation. Unfortunately the pathogenic mechanisms involved in the evolution of this condition are understood poorly. Current evidence suggests that this condition is the result of an acquired inflammatory process that preferentially targets the neonatal biliary tract.[1] A variety of nonspecific initiating events, possibly including neonatal Reovirus type III infection, may trigger this process.[10-12]

This study was designed to develop a model for the study of biliary tract inflammatory processes such as this. It is well recognized that neutrophils and other inflammatory effector cells may participate in the generation of host tissue injury in a variety of experimental and clinical situations.[15-18] Phorbol myristate acetate is a synthetic phorbol ester known to activate phagocytic cells[19-21] as



FIGS. 4a–c. Histologic alterations in the liver of animals given PMA infusions for 28 days. At the time fibrosis was prominent with the development of cirrhosis. In addition there was disordered proliferation of bile duct epithelium (neocholangiogenesis) (a, arrows). Some ongoing chronic inflammation was present, but it was less intense than at the early time points and prominent cholestasis was present (b and c) (hematoxylin and eosin stain; a, 100×; b, 200×, and c, 400×).



FIGS. 5a and b. Comparative histologic alterations in patients with biliary atresia. In the early stages of the disease, there is prominent periportal inflammation with destruction of the bile ducts (a). In the chronic stages of the disease, fibrosis predominates with dense bands of collagen, micronodular cirrhosis, and disordered bile duct proliferation (neocholangiogenesis), as illustrated in b (hematoxylin and eosin stain, 200×).

well as endothelial cells *in vitro*.[22] This process is the result of the activation of protein kinase C, a proximal and therefore nonspecific step in the process of signal transduction in phagocytic cells. In particular PMA induces the oxidative burst in phagocytic cells and in fact, often serves as the laboratory standard to provide maximally activated phagocytic cells. Phorbol myristate acetate-stimulated neutrophils have been implicated in experimental models of inflammatory pulmonary injury.[23–26] Importantly PMA itself is not toxic in neutrophil-depleted experimental systems,[23] in systems where neutrophil adherence to endothelial cells is prevented,[26] or in the presence of neutrophils derived from patients with chronic granulomatous disease (CGD) in whom granulocytes are intrinsically incapable of generating oxygen radicals.[23] Thus it appears that PMA itself is not toxic, but that when administered in the presence of normally functioning neutrophils, injury results.

Reactive oxygen products are widely implicated in a variety of pathologic processes mediated by the endogenous inflammatory response. These products include the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), the hydroxyl radical ($^{\cdot}OH$), and other reactive intermediates generated during the oxidative burst by phagocytic cells activated by PMA. For this reason PMA was selected to induce the biliary inflammatory process in this study. It appears that the resultant tissue injury in this model is associated with an acute and chronic inflammatory process leading to fibrosis of portions of the biliary tree. The histologic features of this injury are similar to those of biliary atresia.

This is a relatively straightforward and inexpensive model that may be useful in the study of pathogenic mechanisms of biliary atresia and other nonpyogenic biliary inflammatory processes. The association of PMA with neutrophil and other phagocytic cell oxidant production suggests that oxidant-mediated tissue injury may play a role in the pathogenesis of this type of biliary inflammation.

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