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# A longitudinal study of histologic and immunohistologic changes in an experimental model of sclerosing cholangitis*

**P. Bedossa [1], S. Houry [2], J. Bacci [1], E. Martin [1], G. Lemaigre [1], and M. Huguier [2]**

[1] Laboratoire d'Anatomie Pathologique, Hopital Antoine Béclère, Clamart, France
[2] Service de Chirurgie Digestive, Hopital Tenon, Paris, France

**Summary.** A longitudinal study of intra and extrahepatic bile duct injuries was performed in an animal model of secondary sclerosing cholangitis induced by formalin injection into the common bile duct. Lymphocytic infiltration inside and around the bile ducts occurred seven days after injection. The disease later evolved to a fibrous cholangitis of the small bile ducts. Septal intrahepatic and extrahepatic bile duct involvement became evident three months after formalin injection. The ductular proliferation led to a progressive biliary cirrhosis with portal to portal fibrous septa. After formalin injection, bile duct cells expressed the Ia antigen in the cytoplasm and/or on the membrane of bile duct cells. The intensity of staining did not correlate with the duration or severity of the disease. Lymphocytes infiltrating into and around the bile duct were mainly T-cells. This study suggests that a local cell-mediated immune response to the injection of a toxic agent induces pathological features similar to those of sclerosing cholangitis in man.

**Key words:** Cholangitis – Major histocompatibility complex antigen – Immunohistochemistry

## Introduction

Sclerosing cholangitis (SC) is an uncommon disease characterized by fibrosing inflammation of the bile ducts (Barbatis et al. 1985; Chapman et al. 1980; International group 1983; Ludwig et al. 1981; Ludwig et al. 1984). It usually affects both the extra and intrahepatic areas of the biliary tree. Selection criteria for SC are those of a long-standing disease including characteristic biological and radiological alterations (Chapman 1985; Larusso et al. 1984; Wiesner and Larusso 1980) and thus the earlier topographical and morphological lesions in SC are not known. The isolated periductal fibrosis reported in patients with ulcerative colitis might be the first step in SC, but the subsequent evolution of this lesion remains unclear (Mistilis 1965; Wee and Ludwig 1985).

The aetiology of SC is unknown but several reports suggest that an immune-mediated mechanism plays a role in the pathogenesis of primary sclerosing cholangitis (Bodenheimer et al. 1983; Lindor et al. 1987; Mac Farlane et al. 1979). This hypothesis has been recently reinforced by immunohistochemical studies showing that bile duct cells in SC express the HLA Class II antigen (Chapman et al. 1988) and that the lymphocytic infiltration around bile duct lesions is mainly composed of T-lymphocytes (Whiteside et al. 1985).

In the present study we report an experimental model of SC in the rat. In this model, radiographic and histological lesions characteristic of SC are present three months after injection of a 2% formalin solution into the common bile duct. We describe the light microscopic features and the longitudinal evolution of the intra and extra hepatic biliary tree lesions. Using an immunohistochemical method, we investigate the expression of the Ia antigen on bile duct epithelium and the lymphocyte subsets in damaged portal areas.

## Materials and methods

36 Sprague-Dawley rats were anesthetized and after a midline abdominal incision, underwent a 2 mm duodenotomy. A catheter was introduced into the common bile duct through the am-

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*Offprint requests to:* P. Bedossa, Laboratoire d'Anatomie Pathologique, Hopital Antoine Béclère 157, rue de la Porte de Trivaux, 92141 Clamart, France

**Table 1.** List and specificities of monoclonal antibodies

| Antibody against | Clone | Origin |
|---|---|---|
| Ia | O × 6 | Sera-lab |
| T-lymphocytes | W3/13 | Sera-lab |
| T helper cells | W3/25 | Sera-lab |
| T non-helper cells | O × 8 | Sera-lab |

pulla. 0.15 ml of 2% formalin solution was injected and the common bile duct was clamped just above the catheter. Five min later, the clamp was removed and the duodenotomy closed.

A control group of 24 age-matched rats were given sterile isotonic solution instead of formalin. Groups of 5 rats (2 from the control group and 3 from the pathological group) were killed on day 1, 2, 3, 7, 15, 30, 45, 60, 90, 180, 270 and 360 after injection.

Samples of the right and left liver lobes and of the common bile duct were removed immediately after killing. Each sample was divided into two parts, one of which was frozen in liquid nitrogen and the other fixed in Bouin solution. Fixed tissue was routinely processed, cut into serial sections and stained with haematoxylin-eosin, Masson's trichrome and reticulin, according to Gordon and Sweet.

The anatomical terms chosen in the study are those usually accepted: "interlobular bile duct" was used for the smallest branches (less than 100 μm in diameter) lined with cuboidal epithelium and always accompanied by an hepatic artery and a portal vein radicle. "Septal bile duct" was used to denote branches lined with cylindrical epithelium, whose diameter usually exceeded 100 μm. "Segmental bile ducts" signified the ducts formed by the merger of the septal bile ducts several centimeters from the hilum. The definitions of morphological manifestations are those currently in use (International group 1983).

*Immunohistochemistry.* The Ia antigen and lymphocyte antigens were localized using monoclonal antibodies (Table 1) and the avidin-biotinylated peroxidase complex (Vector labs, Burlingame, California). Briefly, serial frozen sections (4 μm thick) were fixed in cold acetone for 10 min, washed in phosphate-buffered saline (PBS) and covered with the purified monoclonal antibody at the appropriate dilution for 30 min. Antibodies were all of the IgG1 subclass and were produced by mouse hybridomas. The avidin-biotin-peroxidase complex technique (Hsu et al. 1981) was used for immunostaining. After washing in three changes of PBS, a biotin-conjugated adsorbed rat anti-mouse IgG was applied prior to the avidin-conjugated horseradish peroxidase (Vector). The sections were then incubated in diaminobenzidine and counterstained with Mayer's haemalum.

For control sections, PBS or non-immunized serum were used as primary antibodies.

## Results

The sequential analysis of livers in the control group showed no significant changes. The only modification was a slight dilatation of the interlobular bile duct lumen in rats killed on day 1, 2 and 3 after injection of isotonic serum. Dilatation or narrowing of the intra or extrahepatic biliary tree were never observed.

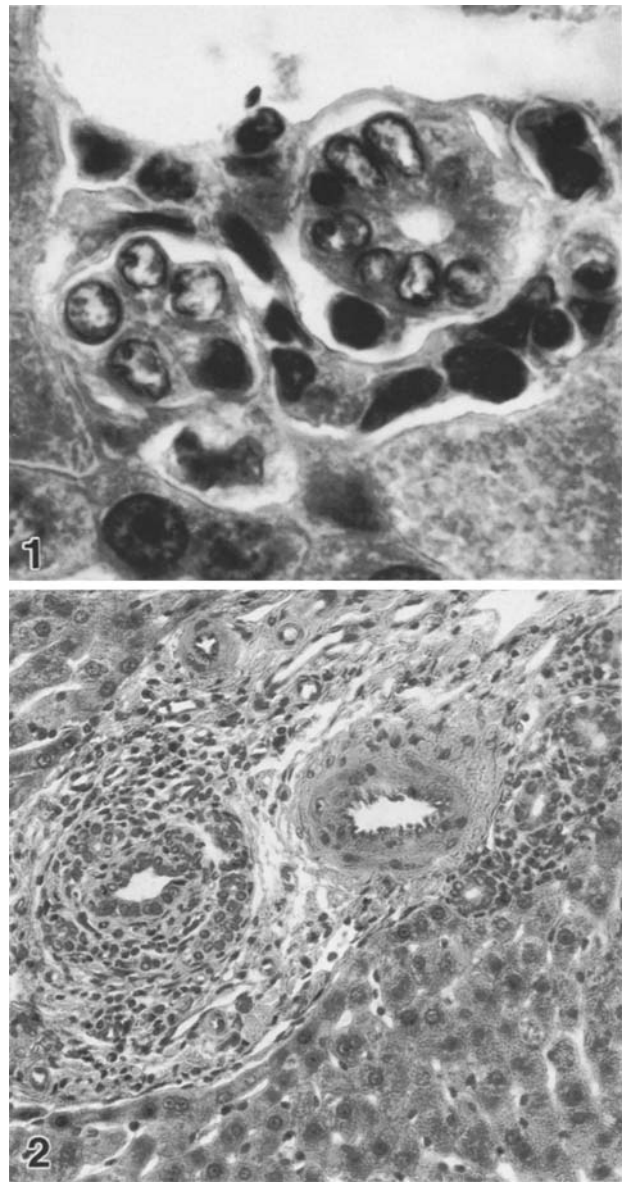


**Fig. 1.** Interlobular bile duct, 7 days after formalin injection. Lymphocytes are present inside and around the bile duct epithelium (HES × 400)

**Fig. 2.** Interlobular bile duct, 2 months after formalin injection. Fibrous cholangitis with moderate mononuclear cell infiltration and ductular proliferation in the periportal area. (HES × 250)

After formalin injection, the earliest changes occurred on day 7 after injection. A mild lymphocytic infiltration in the portal tracts was noted with, in some cases, lymphocytes inside the epithelial layer of the bile ducts (Fig. 1). No acute necrosis of the bile duct epithelium was noted but clarification of the cytoplasm and abnormal nuclear orientation were frequent. Hepatic lobules and central veins displayed no abnormalities.

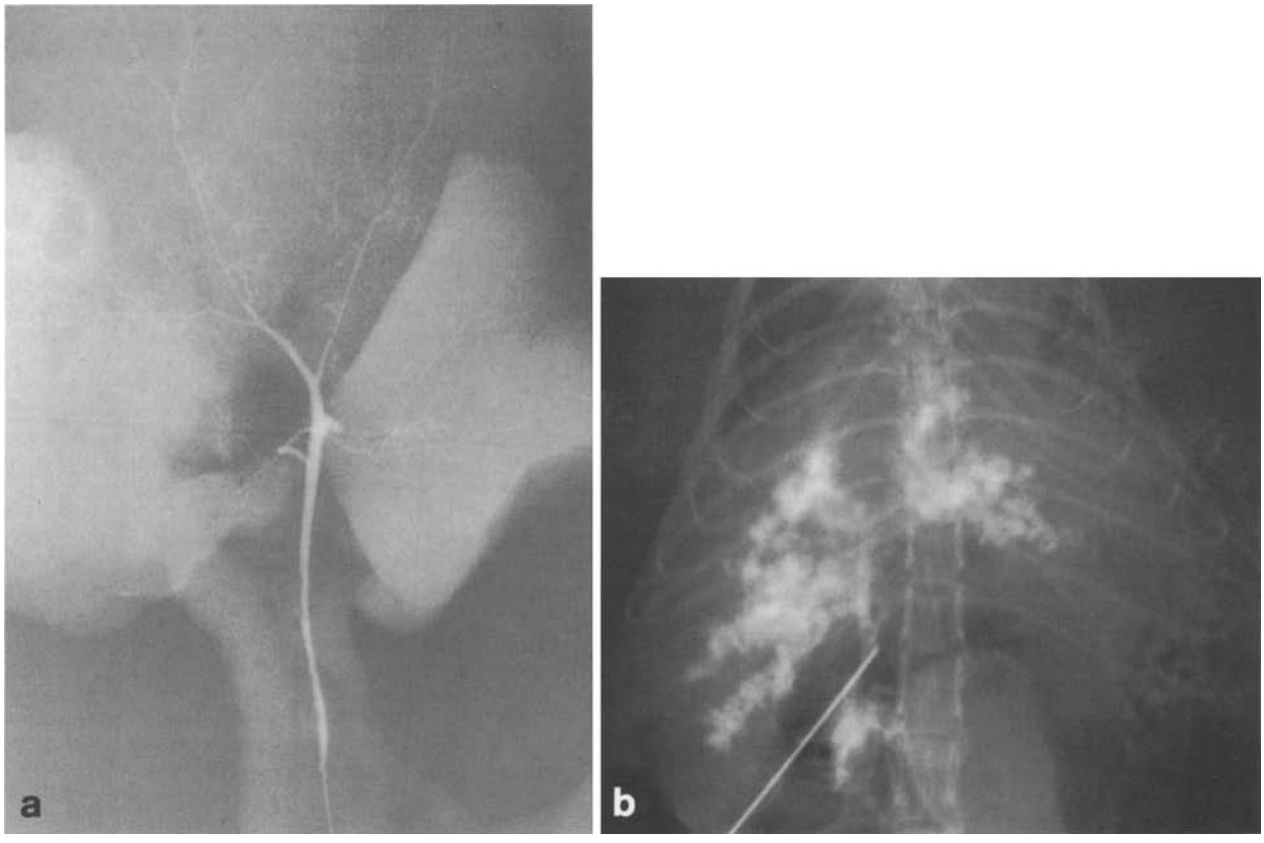


**Fig. 3a, b.** Retrograde cholangiogram. **a** Cholangiogram from rat of the control group killed three months after isotonic serum injection. **b** Three months after injection of formalin. Note the irregular beading and strictures of the intra and extrahepatic bile ducts

Rats sacrificed on days 15 and 30 after formalin injection also displayed non specific epithelial changes in the interlobular bile duct cells. Neither fibrous cholangitis nor obliterative fibrous cholangitis were noted. Portal tracts contained many fibroblasts, macrophages and lymphocytes with a few polymorphonuclear leukocytes. Venules and lymphatics were slightly dilated. No changes were observed in the hepatic lobule. Extrahepatic bile ducts were normal or showed a mild lymphocytic infiltrate in the submucosa.

On day 60 after injection of formalin, pleomorphic and fibrous cholangitis of the interlobular bile ducts with mild-to-moderate obliteration was observed. Moderate periductal lymphocytic infiltration and proliferation of ductules were present (Fig. 2). Septal and extrahepatic bile ducts were intact.

Advanced lesions, three months after injection, were characterized by distal and proximal alterations of the biliary tree (Fig. 3). Lesions included fibrous obliterative cholangitis (Fig. 4a) with occasional total destruction of interlobular bile ducts and the formation of a nodular scar (Fig. 4b). The inflammatory infiltrate decreased. In the inner part of the parenchyma near the hilum, numerous tubular and saccular cholangiectases were noted. They were characterized by large dilated septal or segmental bile ducts with semicircular crests. The epithelial lining was elongated or had been destroyed (Fig. 4c). In the portal tracts, fibrous deposits, ductular proliferation and excessive dilatation of lymphatics occurred. Lobular changes included cholestasis, Kupffer cell hyperplasia and a mild piecemeal necrosis.

In extrahepatic bile ducts, pathological features consisted of flattening of epithelial cells lying on a loose fibrous tissue layer with mild chronic inflammation. Some, but not all of the vessels of the submucosae displayed a mild degree of periarteritis or a hyaline thickening of the media. Total occlusion of the vascular lumen was never present (Fig. 5).

Nine months after surgery, portal tract fibrosis expanded into the lobule with portal to portal thin fibrous septa. One year after formalin injection, biliary cirrhosis was evident.

The results of immunocytochemical investiga-

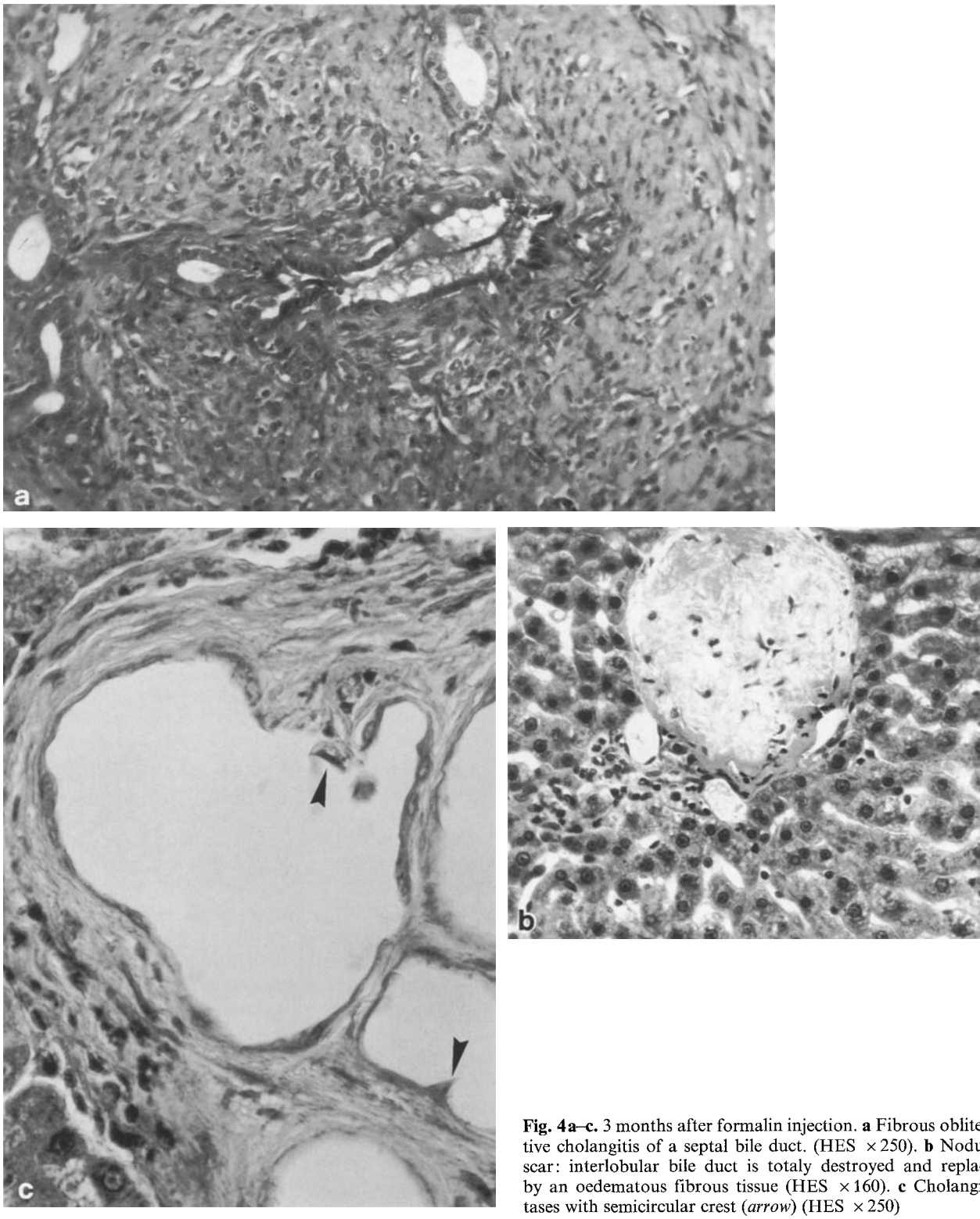


**Fig. 4a–c.** 3 months after formalin injection. **a** Fibrous obliterative cholangitis of a septal bile duct. (HES ×250). **b** Nodular scar: interlobular bile duct is totaly destroyed and replaced by an oedematous fibrous tissue (HES ×160). **c** Cholangiectases with semicircular crest (*arrow*) (HES ×250)

tion showed that in control rat liver Ia antigen expression was restricted to the sinusoidal lining cells and to a few spindle cells and macrophages in the portal tracts. Bile duct cells never expressed Ia antigen after isotonic solution injection.

After formalin injection Ia antigen was expressed on bile duct epithelium from day 7 after injection. The Ia antigen was expressed in the cytoplasm and/or on the membrane of cells of some bile ducts (Fig. 6), whilst other bile ducts were

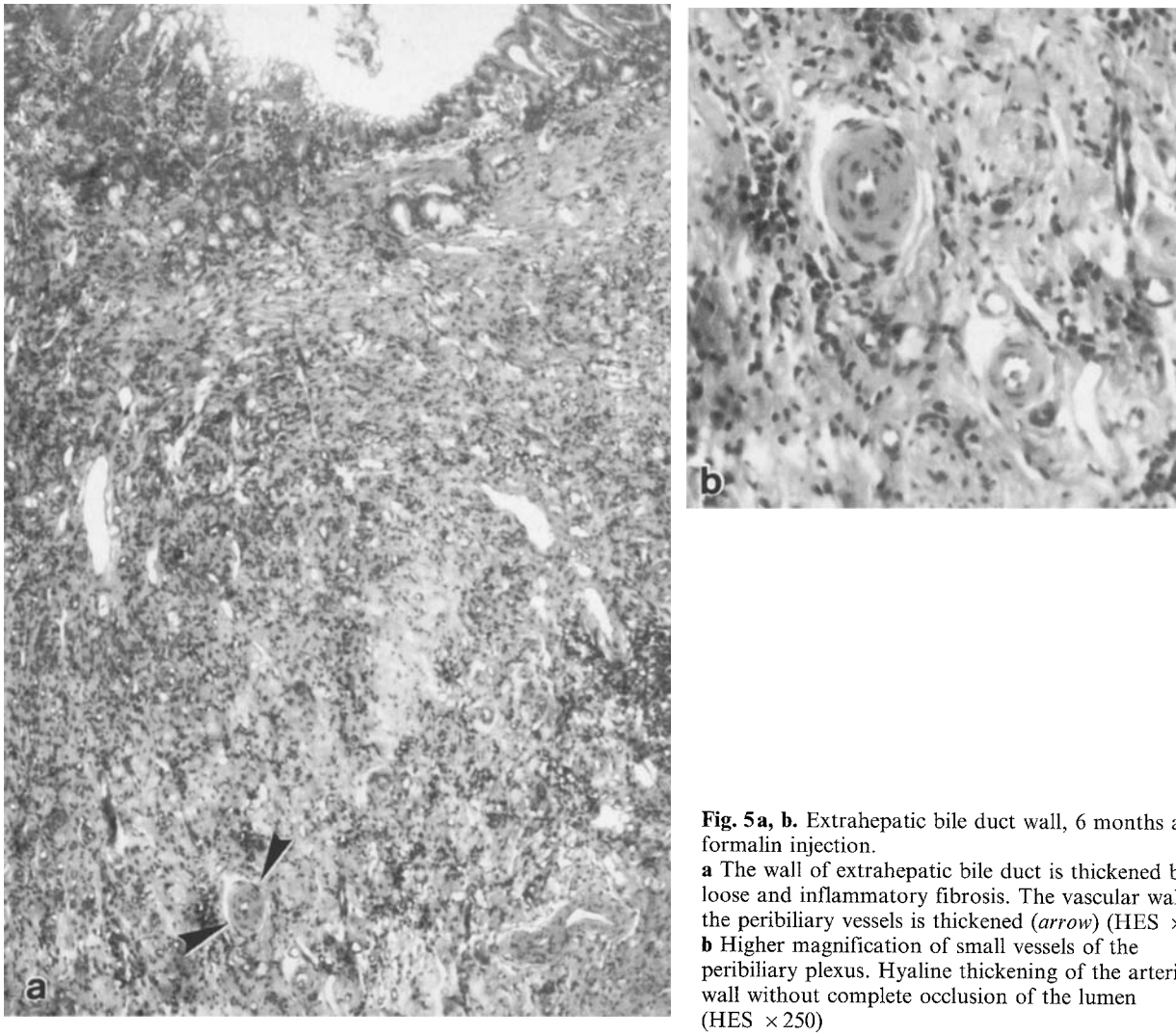


**Fig. 5a, b.** Extrahepatic bile duct wall, 6 months after formalin injection.
**a** The wall of extrahepatic bile duct is thickened by a loose and inflammatory fibrosis. The vascular wall of the peribiliary vessels is thickened (*arrow*) (HES ×60).
**b** Higher magnification of small vessels of the peribiliary plexus. Hyaline thickening of the arterial wall without complete occlusion of the lumen (HES ×250)

completely negative. Staining intensity did not correlate with the duration and the severity of the lesions. The Ia antigen expression was not modified in the lobules after formalin injection.

The amount of mononuclear cell (MNC) infiltrate varied widely according to the stage of the disease, with numerous MNC in the early inflammtory phase and with a few MNC at the later fibrous stage of the disease. T-cells were the predominant cells infiltrating into and around the bile ducts (Fig. 7) forming at least 80% of the MNC, but the percentage of the T-cell subtypes varied according to the portal tract. T-helper cells predominated in the infiltrates and in cells invading the bile duct but T suppressor cells were also increased around the ducts, often in close contact with them.

## Discussion

Although histological findings do not permit diagnosis of SC, a wide range of interlobular bile duct abnormalities has been found. Ludwig et al. (1986), who studied fresh whole liver from patients with primary sclerosing cholangitis at the time of transplantation, provided a morphological description of medium and large bile ducts in SC. These authors consider that the combination of cholangiectases and intrahepatic bile duct obliteration is pathognomonic of sclerosing cholangitis. As we observed similar lesions in this study, we may justifiably conclude that the present surgical procedure provides a valid experimental model of sclerosing cholangitis. Furthermore, the absence of acute inflammation or bile duct epithelium necrosis and the shape and distribution of the lesions lead us to exclude a suppurative cholangitis or passive dilatation of previously normal duct segments as causative factors. To our knowledge, this is the only experimental model of sclerosing cholangitis. However, as the formalin injection used here is the predisposing factor, the term secondary scler-


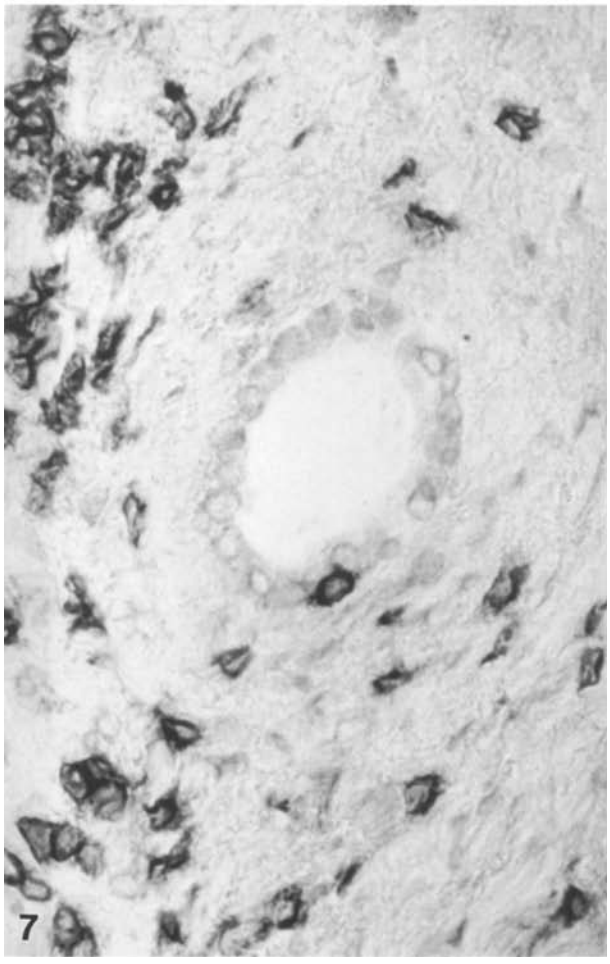


**Fig. 6.** Immunostaining of the Ia antigen in a bile duct. Epithelial cells display immunoreactivity on membrane and cytoplasm. (Immunostaining × 250)

**Fig. 7.** Demonstration of T-lymphocytes in a portal tract, 15 days after formalin injection. Note the presence of an intra-epithelial T-cell. (Immunostaining × 320)

osing cholangitis must be used for the present disorder.

In this rat model, early lesions are located in the interlobular bile duct, far from the site of formalin injection, whereas the entire distance between interlobular bile duct and Vater's papilla is involved by the end stage of the disease. In man, topographical and morphological data concerning early lesions in sclerosing cholangitis are not available. Liver biopsies from patients with chronic ulcerative colitis show the high frequency of isolated intrahepatic bile duct lesions, which are indistinguishable from primary sclerosing cholangitis; some of these patients develop typical radiological sclerosing cholangitis with extrahepatic bile duct involvement a few years later (Blackstone and Nemchausky 1978; Wee and Ludwig 1985). Our results are consistent with such an evolution in this experimental model.

The mechanism that triggers fibrogenesis is unknown. In human, secondary SC has been reported after intra-arterial chemotherapy (Kemmeny et al. 1985; Shea et al. 1986). The pathogenesis is unclear but an ischaemic mechanism is thought to be involved (Marymont et al. 1985). Although the injection route of formalin is different here, we also noted slight vascular lesions of peribiliary vessels but we have never observed complete occlusion whatever the stage of the disease. Thus, while we cannot exclude an ischaemic mechanism, we do not think that this is the essential pathogenic mechanism.

Our study is consistent with others in human SC (Barbatis et al. 1985; Nakanuma et al. 1986) in showing a close relationship between bile duct cells and lymphocytes during the early stages of the disease. Furthermore the presence of numerous T-cells in the infiltrate as well as into the bile duct suggests that, in the present model, cellular immunity has a predominant part in the reaction triggered by the formalin injection.

In man, the emergence of the expression of the major histocompatibility complex (MHC) class II antigen on epithelial cells is characteristic of several auto-immune diseases, for example, diabetes (Bottazo et al. 1985), Graves disease (Hanafusa et al. 1983) or primary biliary cirrhosis (Ballardini et al. 1987). MHC class II expression enables the cells to present auto-antigens to T-lymphocytes which may participate in the immune response. Such an aberrant expression also has recently been reported on bile duct epithelium in primary SC (Chapman et al. 1988, Van Den Oord et al. 1986) drawing a parallel with an auto-immune process. The significance of aberrant MHC Class II expres-

sion by bile duct cells is, in fact, not pathognomonic of an auto-immune process: similar expression is reported in non-immune cholestatic diseases or in chronic or acute cholangitis (Chapman et al. 1988; Van Den Oord et al. 1986). These results are in accord with our observation in a non auto-immune model of SC, and suggest therefore that MHC Class II expression is a secondary phenomenon to inflammation or cholestasis rather than a primary event. MHC Class II product synthesis might be induced by lymphocytes because intra- und peri-ductal lymphocytes are a common finding and because it has been demonstrated that in the gut, intra-epithelial lymphocytes modulate the Ia expression on epithelial cells (Cerf-Bensoussan et al. 1985).

Lastly, this study shows that formalin injection induces SC. Light microscope study and immunohistochemical results suggest that cellular immunity is involved. The aberrant expression of MHC Class II on bile duct cells might be a secondary phenomenon in SC.

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