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Histologic Studies on the Hepatic Lesions Induced by Graft-Versus-Host Reaction in MHC Class II Disparate Hosts Compared with Primary Biliary Cirrhosis

Tadao Saitoh,*† Michio Fujiwara,* Minoru Nomoto,† Tomoteru Kamimura,† Kiyoshi Ishihara,‡ and Hitoshi Asakura†

From the Department of Immunology and the Third Department of Internal Medicine,† Niigata University School of Medicine, and the College of Biomedical Technology, Niigata University,‡ Niigata, Japan*

By light and electron microscopic examinations, histologic changes in the liver of mice with graft-versus-host reaction (GVHR) were analyzed. To induce GVHR, C57BL/6 (B6) spleen cells were injected into (B6×bm1)F1, (B6×bm12)F1, and (bm1×bm12)F1 mice. In (B6×bm12)F1 recipient mice, bile duct changes resembling chronic nonsuppurative destructive cholangitis (CNSDC) and a formation of epithelioid granulomas were observed during the course of GVHR. An epithelioid granuloma in the liver of (B6×bm1)F1 or (bm1×bm12)F1 recipients was not detected. By electron microscopy, the bile duct epithelia were seen to be in close contact with infiltrating cells, and marked alterations of their cytoplasm and microvilli were demonstrated; ie, vacuolation of the cytoplasm, deterioration of microvilli, and bleb formation were frequently observed in the liver of class II-disparate hosts. Concerning the basement membrane, no marked changes characteristic of primary biliary cirrhosis (PBC), such as many-layered basement membranes containing osmium positive substance, were detected. Because the major histocompatibility complex (MHC) class II-disparate system was used in our experimental system in the GVHR, the antigen expressed on the bile duct might be a target and be associated with the formation of the initial hepatic lesions in PBC such as CNSDC and epithelioid granuloma formation. Thus, GVHR across the MHC class II antigen is believed to play an important role in the development of PBC. (Am J Pathol 1989, 135:301–307)

Primary biliary cirrhosis (PBC) is characterized at an early stage by chronic nonsuppurative destructive cholangitis (CNSDC), cholestasis, and epithelioid granuloma formation.[1,2] Many infiltrating cells were shown to consist of mononuclear cells, such as lymphocytes, plasma cells, or eosinophils. Ultrastructural studies of the bile ducts of patients with PBC also showed that lymphocytes at an early stage were frequently present within spaces among necrotic biliary epithelial cells.[3,4] In addition, serum levels of antibodies against mitochondria, smooth muscles, and nuclear, and hepatobiliary antigens were shown to be elevated in the disease.[5,6] Based on these findings, a role for an autoimmune mechanism in the destruction of bile duct epithelial cells in PBC has been suggested. Etiopathogenesis of the disease has not been established yet, but one of the favored hypotheses is that the degenerative and destructive changes of the bile ducts may be caused by T cells mediating a chronic graft-versus-host reaction (GVHR).[7]

GVHR against histocompatibility antigens is known to cause hepatic lesions in the portal area at the level of interlobular bile ducts. Recently, we demonstrated that the bile duct lesions resembling CNSDC and the formation of epithelioid granuloma in the GVHR were induced with differences of the MHC class II antigens.[8] However, in the MHC class I- or class I plus class II-disparate GVHR, no epithelioid granuloma was found, although changes in the bile duct epithelium, including the basement membrane in the MHC class I plus II GVHR, were more severe than those in the MHC class II-disparate GVHR. In this article, light microscopic and ultrastructural findings of the bile duct epithelium and cellular infiltrations in the portal area in mice with MHC class II or class I plus class II GVHR are described. The findings are discussed in comparison with the morphologic features of PBC.

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Address reprint requests to Dr. Tadao Saitoh, Department of Immunology, Niigata University School of Medicine, Asahimachi 1, Niigata 951, Japan.

Table 1. *Haplotypes of H-2 Regions of Mouse Strains Used*

Strains	Alleles at H-2 regions			
	K	I-A	I-E	D
C57BL/6 (B6)	b	b	—	b
B6.C-H-2^{bm1} (bm1)	bm1	b	—	b
B6.C-H-2^{bm12} (bm12)	b	bm12	—	b
(B6×bm1)F1*	b/bm1	b	—	b
(B6×bm12)F1*	b	b/bm12	—	b
(bm1×bm12)F1*	b/bm1	b/bm12	—	b

* These F1 hybrid mice, (B6×bm1)F1, (B6×bm12)F1, and (bm1×bm12)F1, that were injected with B6 spleen cells, were found to cause MHC class I-, class II-, and class I plus II-disparate GVHR, respectively.

Materials and Methods

Mice

Murine strains used were C57BL/6 (B6), B6 mutant B6.C-H-2^{bm1} (bm1), which carries a mutant gene at the *H-2K* locus,[9] and B6.C-H-2^{bm12} (bm12), which carries a mutant gene at the *I-A* locus of MHC.[10] These mice originated at the Jackson Laboratory, Bar Harbor, ME. The bm1 strain was provided by Dr. T. Nishizawa (Department of Dental Research, National Institute of Health, Tokyo, Japan) and the bm12 strain by Dr. H. Ishikawa (Department of Microbiology, Keio University School of Medicine, Tokyo, Japan). F1 hybrid mice, (B6×bm1)F1, (B6×bm12)F1, and (bm1×bm12)F1, were bred at our own animal facilities. The genetic differences of these mice are shown in Table 1.

Preparation of Donor Cells and the Induction of GVHR

Five-to-six mice were used for each experimental group. To induce GVHR, a normal B6 spleen cell suspension was prepared in Eagle's minimum essential medium (MEM, Nissui, Tokyo) containing 2% fetal calf serum (FCS, Bockneck, Rexdale, Ontario, Canada), buffered with 5 m*M* HEPES (Sigma Chemicals, St. Louis, MO), washed in the medium without FCS, and injected into each F1 hybrid mice at 5×10^7 cells in two equal dosages on days 0 and 7.[8,11] These mice were killed at 2, 4, 6, 8, and 10 weeks after the first inoculation of donor cells.

Enumeration of Immunoglobulin-Producing Cells

The total number of IgM- and IgG-secreting cells in spleen was determined by the reverse plaque assay in a Cunningham chamber using protein A coated sheep red blood cells.[8]

Histology

Liver specimens were prepared from each mouse and divided in two. One specimen was fixed in 10% phosphate-buffered formalin, embedded in paraffin, and used for light microscopic study. For each specimen, five-to-six portal areas were enumerated, and all inflammatory cells infiltrating into the portal tracts were counted at a magnification of 200 under a microscope equipped with a micrometer reticle (0.25 mm square, Olympus, Tokyo). The lesions were graded by an average number of infiltrating cells in five or six portal tracts chosen at random as follows: grade 0, none; grade 1, mild (less than 100); grade 2, moderate (100 to 400); grade 3, marked (more than 400). The other specimen was fixed in 2.5% glutaraldehyde, postfixed in 2.0% osmium tetroxide, dehydrated in graded ethanol, and embedded in Epon 812 for electron microscopy. Ultrathin sections were made by an ultramicrotome, stained with uranyl acetate and lead citrate, and examined under an electron microscope (JEOL 1200 EX, Nihondenshi, Tokyo).

Results

Mice were killed for the histopathologic examinations at various weeks after B6 spleen cells were injected into F1 hybrid mice. All (B6×bm1)F1 and (B6×bm12)F1 mice with GVHR looked well during the observation period of 10 weeks, but (bm1×bm12)F1 recipients began to die with symptoms of progressive weight loss, ruffled fur, and alopecia at approximately 4 weeks after injection of B6 spleen cells. Mortality by 6 weeks after injections was 20% to 60%, depending on each experimental condition. In (B6×bm12)F1 recipients injected with B6 spleen cells that induced class II GVHR, immunoglobulin-producing cells in the spleen increased in number at 2 weeks after injection, an increase that was sustained for several weeks (data not shown).[8] This indicated B cell activation in GVHR.

Histologic studies by light microscopy in the MHC class II GVHR revealed conspicuous findings in the portal area at the level of the small interlobular bile ducts and around the central vein of the liver. As shown in Figure 1A, moderate-to-marked infiltrations of inflammatory cells consisting of lymphocytes, plasma cells, and eosinophils had been observed in the portal area at the 2nd week. At this time, bile duct lesions resembling CNSDC in PBC began to appear. These lesions were constantly observed up to 10 weeks postinjection. Some epithelioid granulomas in our experimental GVHR, which have not previously been described in the literature, were observed in the portal area and also around the central vein (Figure 1B).

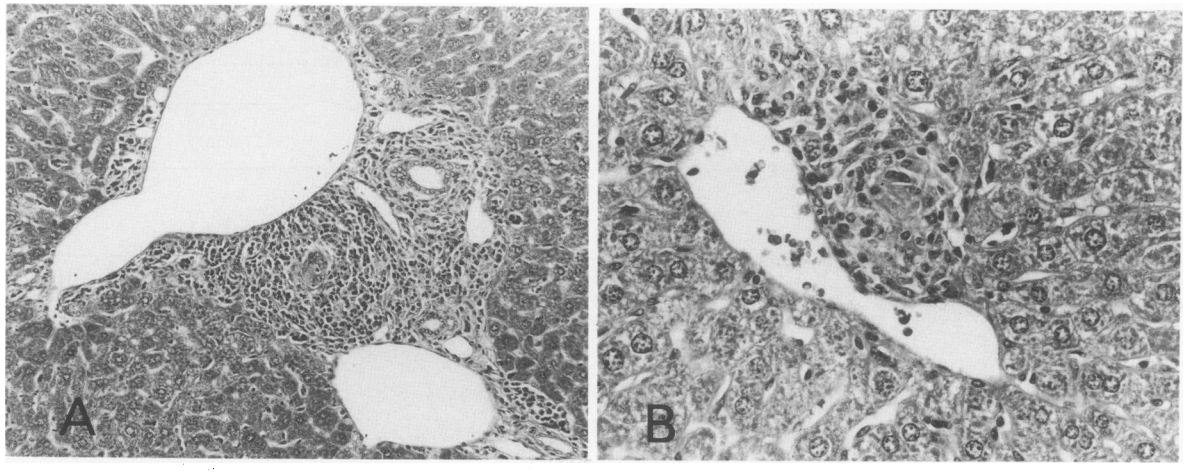

Figure 1. *Light microscopic appearance of the hepatic lesions stained by hematoxylin and eosin in (B6×bm12)F1 mice injected with B6 spleen cells.* **A:** *At 8 weeks after injection, the specimen showed a marked cell infiltration and bile duct changes resembling CNSDC in PBC (H & E, ×100).* **B:** *An epithelioid granuloma formation around the central vein at 8 weeks (H & E, ×200).*

Similar or severer inflammatory lesions of the bile duct were also observed in (bm1×bm12)F1 mice with the MHC class I plus class II GVHR (Figure 2A). At the 5th or 6th week after injections, periductal fibrosis and sparse inflammatory cell infiltrations were observed (Figure 2B), but no epithelioid granuloma had been detected in (bm1-×bm12)F1 recipients of B6 spleen cells throughout the course of GVHR. In (B6×bm1)F1 mice, although slight-to-moderate degrees of cell infiltrations were observed at 2 weeks after injection of B6 spleen cells, these changes disappeared by week 4 or 5. These light microscopic findings are summarized in Table 2.

Electron microscopy in (bm1×bm12)F1 recipients revealed degenerative bile duct epithelia and infiltrating lymphocytes among the bile duct cells. Similar changes, although less noticeable, were also observed in (B6-×bm12)F1 recipients. As shown in Figure 3A, many mononuclear cells, consisting mainly of lymphocytes, surrounded the bile duct in closer contact with the epithelial cells, some of which infiltrated into the intercellular space of bile duct epithelia. Marked cytoplasmic vacuolation, deterioration of microvilli, and bleb formation of the bile duct cells were also demonstrated (Figure 3B). In (bm1-×bm12)F1 recipients, a moderate degree of periductal fibrosis around the bile duct was observed at the week 10 (Figure 4A). This finding contrasted to that observed in (B6×bm12)F1 recipients in which continued cell infiltrations were observed. Occasionally myelin-like substances were detected in the cytoplasm of both recipients (Figure 4B). These cytoplasmic and villous changes were also observed in human cases of PBC and GVHR after bone marrow transplantation (BMT).[12] In PBC, the irregu-

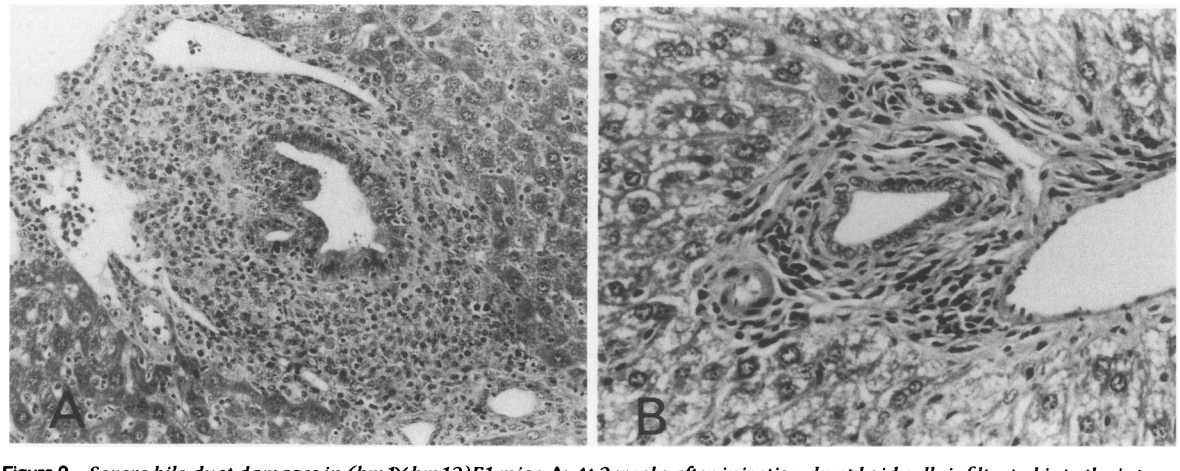

Figure 2. *Severe bile duct damages in (bm1×bm12)F1 mice.* **A:** *At 2 weeks after injection, lymphoid cells infiltrated into the intercellular space of the bile duct epithelia (H & E, ×200).* **B:** *Periductal fibrosis and sparse lymphoid cell infiltrate at 6 weeks (H & E, ×400).*

Table 2. *Cellular Infiltrations in the Portal Area and the Formation of Epithelioid Granuloma*

	Weeks after the injection of B6 spleen cells					
	2 weeks		4 weeks		8 weeks	
Recipient	Cellular* infiltration	Granuloma†	Cellular infiltration	Granuloma	Cellular infiltration	Granuloma
(B6×bm1)F1	2	−	2	−	0	−
(B6×bm12)F1	2	+	2	+	2	+
(bm1×bm12)F1	3	−	2	−	1	−

* Grade of cellular infiltration. Infiltrating cells were enumerated in five or six portal tracts and graded as follows: grade 0, none; grade 1, mild (<100); grade 2, moderate (100 to 400); grade 3, marked (>400). Counting methods were described in detail in Materials and Methods.
† Presence of epithelioid granuloma in the portal area was assessed as negative (−) and positive (+).

larity in the basement membrane, including the many-layered changes and osmium positive substances,[3] was frequently found, but in mice with GVHR, these severe damages were not detected.

Discussion

In this article, histopathologic features of hepatic lesions induced by GVHR using F1 hybrid mice with the differences of the MHC class II antigens were reported. There were surprising similarities between hepatic lesions, especially in the formation of epithelioid granuloma and CNSDC, in PBC and those induced by class II-disparate GVHR. In the latter case, epithelioid granuloma formation, as well as CNSDC, was observed in the portal area and around the central vein by light microscopy. By electron microscopy, many mononuclear cells, mainly lymphocytes, were found in the interlobular bile duct and were present in closer contact with the epithelium. Some were detected in the intercellular space among epithelial cells, which were often accompanied by degenerative changes such as vacuolation, deterioration of microvilli, and bleb formation of the bile duct cells. These are findings that are also frequently found in PBC.[12] On the other hand, there are distinct differences between the hepatic lesions found in GVHR in MHC class II-disparate F1 hybrid mice and those found in PBC in humans. In the latter, hepatobiliary changes containing osmium positive substances are often detected in the basement membrane of the bile duct, but in the former, these severe changes could not be detected. These findings differed to some extent from those described in reports on hepatic lesions after bone marrow transplantation by Bernuau et al.[3] Although the cause of these differences is still unknown, there a difference may exist between PBC- and MHC class II-disparate GVHR-induced lesions in the formation of antibodies against some components of the basement membrane that may be involved in the destruction of the bile duct. We are now investigating autoantibodies, including antibasement membrane antibodies, in GVHR.

A number of reports indicate that hepatic lesions are induced after GVHR both in human cases of bone marrow transplantation and in experimental animals. However, PBC-like lesions have not often been reported in experimental animals. Our studies in mice are conspicuous histopathological report demonstrating hepatic lesions quite resembling with PBC in mice. Epithelioid granuloma formation, a characteristic feature of PBC at an early stage, was observed only in MHC class II-disparate GVHR. We also examined GVHR across the MHC class I and class I plus II antigens. In both cases, CNSDC was observed without an epithelioid granuloma formation.

The immunologic characteristics of the host after induction of GVHR are known to be different according to MHC disparity. As reported by others,[13] autoimmune-like diseases such as systemic lupus erythematosus appear in class II GVHD. In the experimental system reported here, we confirmed the appearance of lupus nephritis with antinuclear antibodies. In addition, histologic changes with lymphoid cell infiltration were observed in the pancreas, lung, salivary glands, and in a part of the small intestines (data not shown). Lymphoid follicles were frequently observed in these organs. These findings are quite compatible with the histologic features of Sicca syndrome, which is supposed to be one of autoimmune diseases frequently complicated by PBC.[7] These light microscopic examinations showed that the histologic changes in the MHC class II-disparate GVHR were surprisingly similar to those in PBC in which autoimmune mechanisms may play a role in the destruction of bile duct cells.

On the other hand, outcomes of class I- or class I plus II-different GVHR were quite unlike those found in class II GVHR. In (B6×bm1)F1 recipients, B cell activation, as assessed by enumerating immunoglobulin-producing cells in the spleen appeared but soon subsided to the level found in normal mice without GVHR.[8] Autoimmune-like diseases were not observed in these cases. In class I plus class II-different hosts, ie, (bm1×bm12)F1 recipients injected with B6 spleen cells, severe immunodeficiency progressed and mice began to die at 4 week after injections.

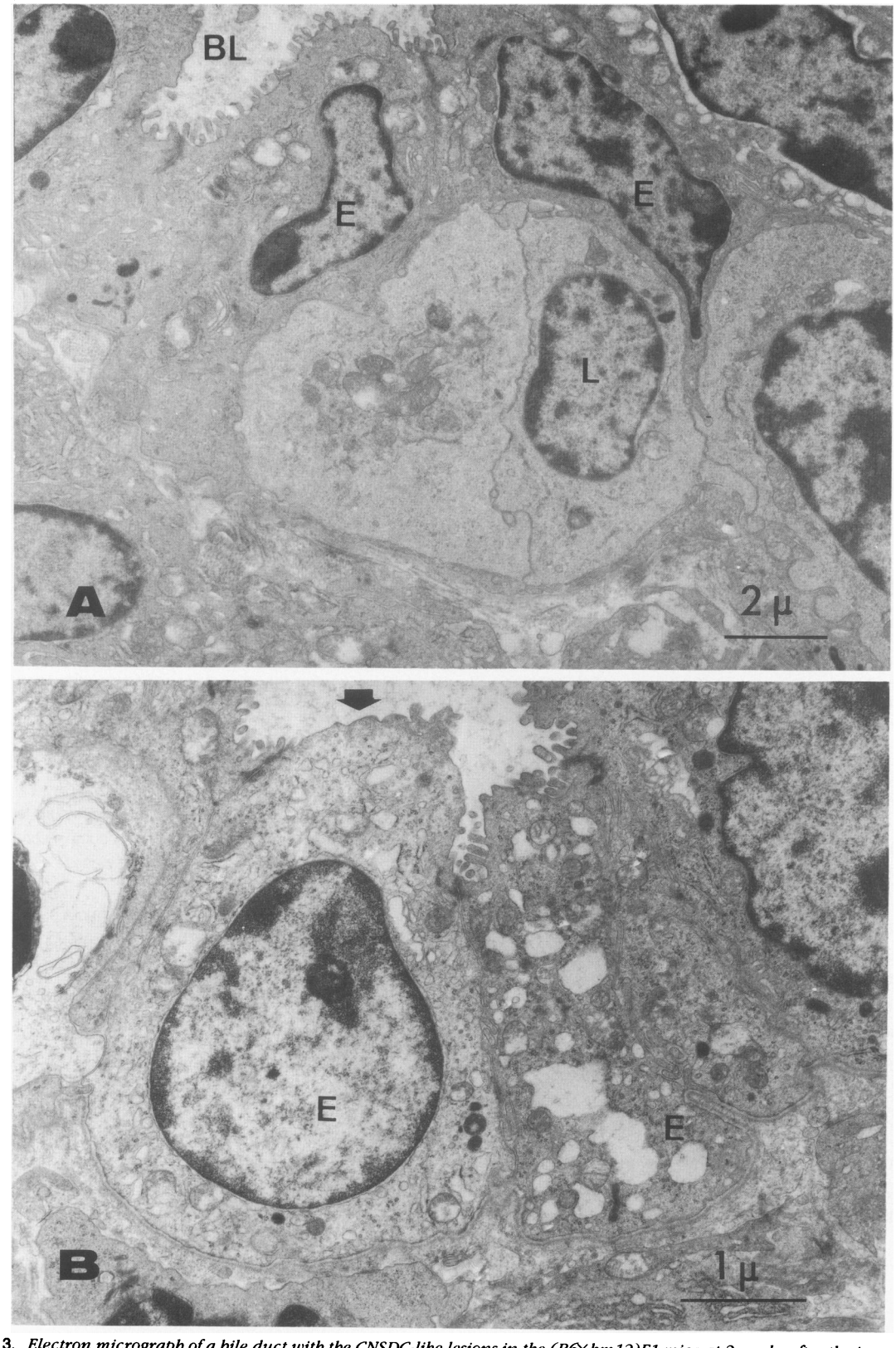

Figure 3. *Electron micrograph of a bile duct with the CNSDC-like lesions in the (B6×bm12)F1 mice at 2 weeks after the inoculation of B6 spleen cells. L, lymphocyte; E, bile duct epithelial cells; BL, lumen of the bile duct.* **A:** *Lymphocytes infiltrated among the epithelia of a bile duct. Note the variation in the nuclear shape and the cytoplasmic vacuolation of the bile duct epithelium. The basement membrane had some degree of change, but remained almost intact without destruction.* **B:** *Marked cytoplasmic vacuolation of the epithelium, and deterioration of microvilli, and the bleb formation (arrow) are observed.*

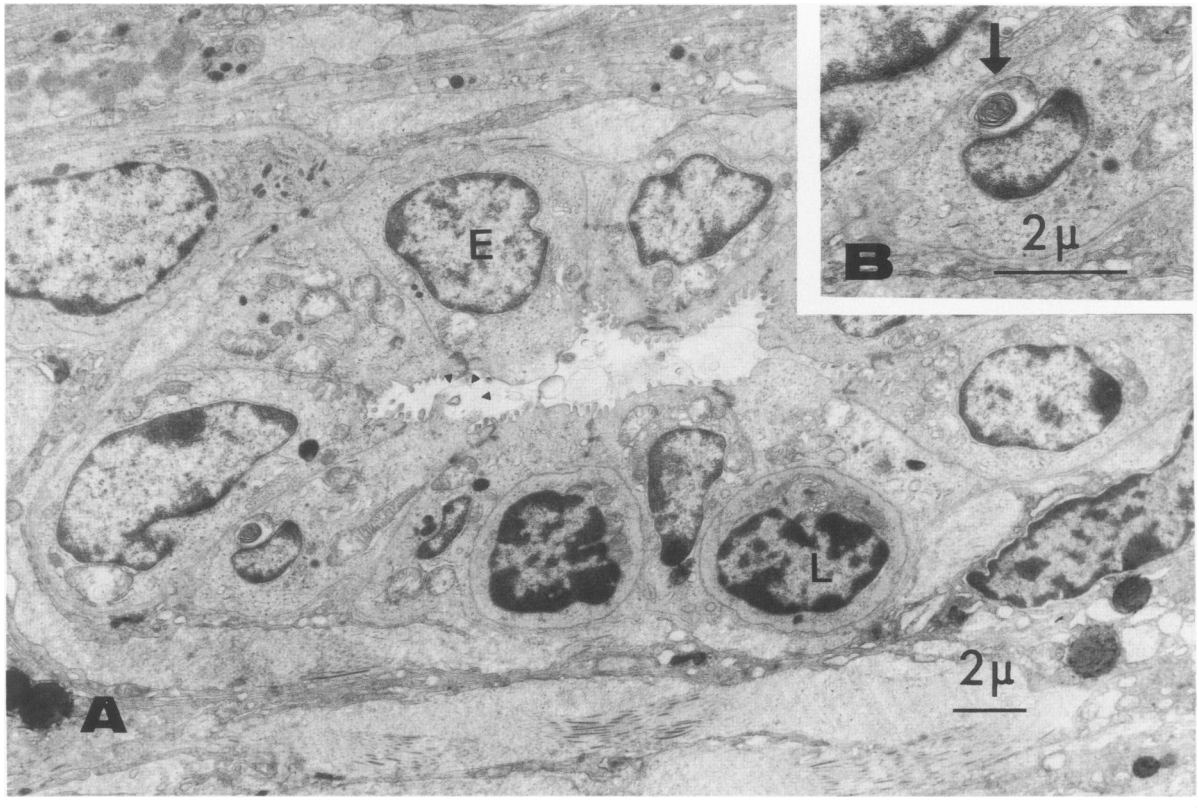

Figure 4. *Electron micrograph of a bile duct of (bm1×bm12)F1 mice at 10 weeks after injection.* **A:** *Lymphocyte infiltration among the epithelia, increased collagen fibers around the bile duct, and alterations of microvilli including the bleb formation (arrow heads).* **B:** *Bile duct epithelium occasionally contained a myelin like substance (arrow).*

In class II GVHR, target cells may be considered to bear Ia antigen(s) and, in fact, we detected the antigen on the damaged bile duct cells (article submitted for publication). Recently, MHC class II antigens were detected in PBC on the damaged bile duct cells,[14] which are known to be induced by lymphokines such as interferon-gamma.[15–18] The expression of MHC class II antigens may be strongly associated with the immune responses, in which $CD4^+$ T cells and/or $CD8^+$ T cells may be activated dependently or independently in their mutual relations and then induce the destruction of bile duct cells and epithelioid granuloma formation. Thus, analyses of infiltrating cells in the hepatic lesions may be important for the understanding of the development of hepatic lesions.

In conclusion, hepatic lesions, the histological features of which resemble those of PBC, were induced in (B6-×bm12)F1 recipients receiving B6 spleen cells. Because sole difference in MHC class II antigen had induced GVHR, it was suggested that PBC might be partly caused during the course of the disease process by T cells recognizing self-Ia molecules.

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