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Modification of swine serum-induced bile duct lesion in BALB/c mice by cyclophosphamide

K. Doi, K. Honjo, C. Doi and T. Mitsuoka
Department of Biomedical Science, Faculty of Agriculture, The University of Tokyo, Bunkyo, Tokyo, Japan

Summary. Effects of cyclophosphamide (CY) on the antibody titer level and incidence and severity of swine serum (SS)-induced bile duct lesion (BDL) were examined. BDL induced by 0.2 ml of SS per head twice a week for 2 weeks was characterized by hyperplasia of biliary epithelial cells, proliferation of mucous glands, and periductal infiltration of eosinophils with mild fibrosis. CY showed no significant influence on the above-mentioned parameters at the dose levels of 140 and 210 mg/kg. On the other hand, CY lowered the antibody titer level and decreased the severity of BDL at the dose level of 280 mg/kg, and it suppressed the antibody response and BDL at the dose level of 280 × 2 mg/kg. Thus the antibody titer level and the severity of BDL were closely related each other.

Key words: Mouse, Swine serum, Bile duct lesion, Cyclophosphamide

Introduction

An eosinophilic and proliferative cholangitis affecting almost the full length of the biliary tract has been induced in mice by repeated intraperitoneal (i.p.) injections of swine serum (SS) for 2 to 4 weeks (Kitamura et al., 1985; Imaoka et al., 1986; Doi et al., 1987; Itagaki et al., 1987, 1988). Recently Glaser et al. (1987) and Fallon-Friedlander et al. (1987) reported that heterologous serum protein might act as an extrahepatic bile duct growth factor in mice. Although they did not refer to any role of recipient's immunological responses in the bile duct enlargement, a participation of anti-SS antibody in the production of bile duct lesion (BDL) has been brought into relief in our previous studies (Imaoka et al., 1986; Doi et al., 1987). Therefore we tried to investigate the SS-induced BDL in mice under different levels of anti-SS antibody titer using cyclophosphamide (CY). CY is a powerful immunosuppressive drug and is being widely used to modify the immunological states of animals (Turk et al., 1972; Jokipii and Jokipii, 1973; Stockman et al., 1973; Kolb et al., 1977; Gaines et al., 1987).

This paper describes the effect of CY on the level of anti-SS antibody titer and on the incidence and severity of SS-induced BDL in mice.

Offprint requests to: K. Doi, Department of Biomedical Science, Faculty of Agriculture, The University of Tokyo, Bunkyo, Tokyo 113, Japan

Materials and methods

Forty-two 8-week-old male BABL/c mice were obtained from SLC Japan Inc. (Shizuoka). Mice were housed in an animal room under controlled condition and fed MF pellets (Oriental Yeast Co., Ltd., Tokyo and water *ad libitum* throughout the experimental period.

They were divided equally into 6 groups (A to F) (Table 1). Group A was given 0.2 ml/head of sterile saline i.p. twice a week on Monday and Thursday for 2 weeks and served as control. Groups B to F were given 0.2 ml / head of sterile SS (Irvine Scientific Inc., CA, Lot No. 6020179) in the same way. At 2 days before the first SS treatment, groups C, D and E were injected i.p. with CY in doses of 140, 210 and 280 mg/kg b.w., respectively. Group F was given 280 mg/kg b.w. of CY i.p. 2 days before the first and third SS treatment. The dose of SS employed in this study was decided based on the data of the preliminary studies on the effect of CY on the lymphoid organs of BALB/c mice.

All mice were killed by exanguination under ether anesthesia 2 days after the last SS treatment. Body and organ (thymus, spleen and liver) weights were recorded and then organ to body weight ratio (g%) was calculated. The thymus, spleen, liver and common bile duct were fixed in 10% neutral buffered formalin. Four μm paraffin sections were stained with hematoxylin and eosin (HE) or periodic acid-Schiff (PAS). The severity of BDL was graded according to Imaoka et al. (1986). To

detect IgG- or IgM-producing lymphocytes, paraffin sections of the spleen were also stained by avidin-biotin-peroxidase complex (ABC) method using Vectastain ABC kit (Vector Laboratories Inc., USA) according to Hsu et al. (1981). Sections stained with normal goat serum instead of anti-mouse IgG or IgM goat serum were examined at the same time.

Serum samples from each mouse were tested for anti-SS antibody (IgG and IgM) titer by the enzyme-linked immunosorbent assay (ELISA) method according to the procedure described in the previous report (Doi et al., 1987). The anti-SS antibody titer of the SS-treated mice was described as the reciprocal of the highest dilution of serum when its optimal dose (OD) value at a wave length of 405 nm was more than 3 standard deviation (s.d.) above the mean of negative serum.

Results

Body and organ weights

Depression of body weight gain was observed in group F. In this group the thymus to body weight ratio was also significantly lower than that of other groups. The spleen weight (g%) of groups B, C, D and F was remarkably higher than that of control group. On the other hand there was no apparent difference in the liver weight (g%) among all groups (Table 1).

Anti-SS antibody titer

As shown in Table 2, the level of IgG antibody titers against SS was similar among 3 groups, B, C and D, whereas it was almost negligible in 2 groups, E and F. The level of IgM antibody titers against SS was also similar among groups B, C, and D, whereas it was very low in group E and was almost negligible in group F.

Histopathological findings

The thymus showed normal histopathological appearance in all groups except in group F. The thymus of group F reduced in size with no change in cortex to medulla ratio.

In the spleen of groups B and C, marked enlargement of lymphoid follicles and prominent increase in number of IgG-positive lymphocytes in the perifollicular and periarteriolar area were noticed (Fig. 1). The number of IgM-positive lymphocytes also increased slightly in the perifollicular area. Although slight depression of follicular lymphocytes was common to the spleen of groups D and E, the spleen of group D had a small cluster of IgG-positive lymphocytes in the periarteriolar area (Fig. 2) while the spleen of group E showed significant decrease in number of IgG- and IgM-positive lymphocytes (Fig. 3) as compared with group A (Fig. 4). Marked depression of both follicular and periarteriolar lymphocytes and prominent enhancement of extramedullary hematopoiesis were observed in the spleen of group F.

As shown in Table 2, the incidence and severity of BDL were almost similar among groups B, C, and D. In comparison with group A (Fig. 5), these groups showed typical BDL which was characterized by hyperplasia of biliary epithelial cells, proliferation of mucous glands, and periductal infiltration of eosinophils with mild fibrosis (Figs. 6, 7). BDL was slight and restricted to the extrahepatic bile duct in group E (Fig 8). In group F, no BDL was observed whereas moderate extramedullary hematopoiesis was found in the liver.

Table 1. Body and organ weights

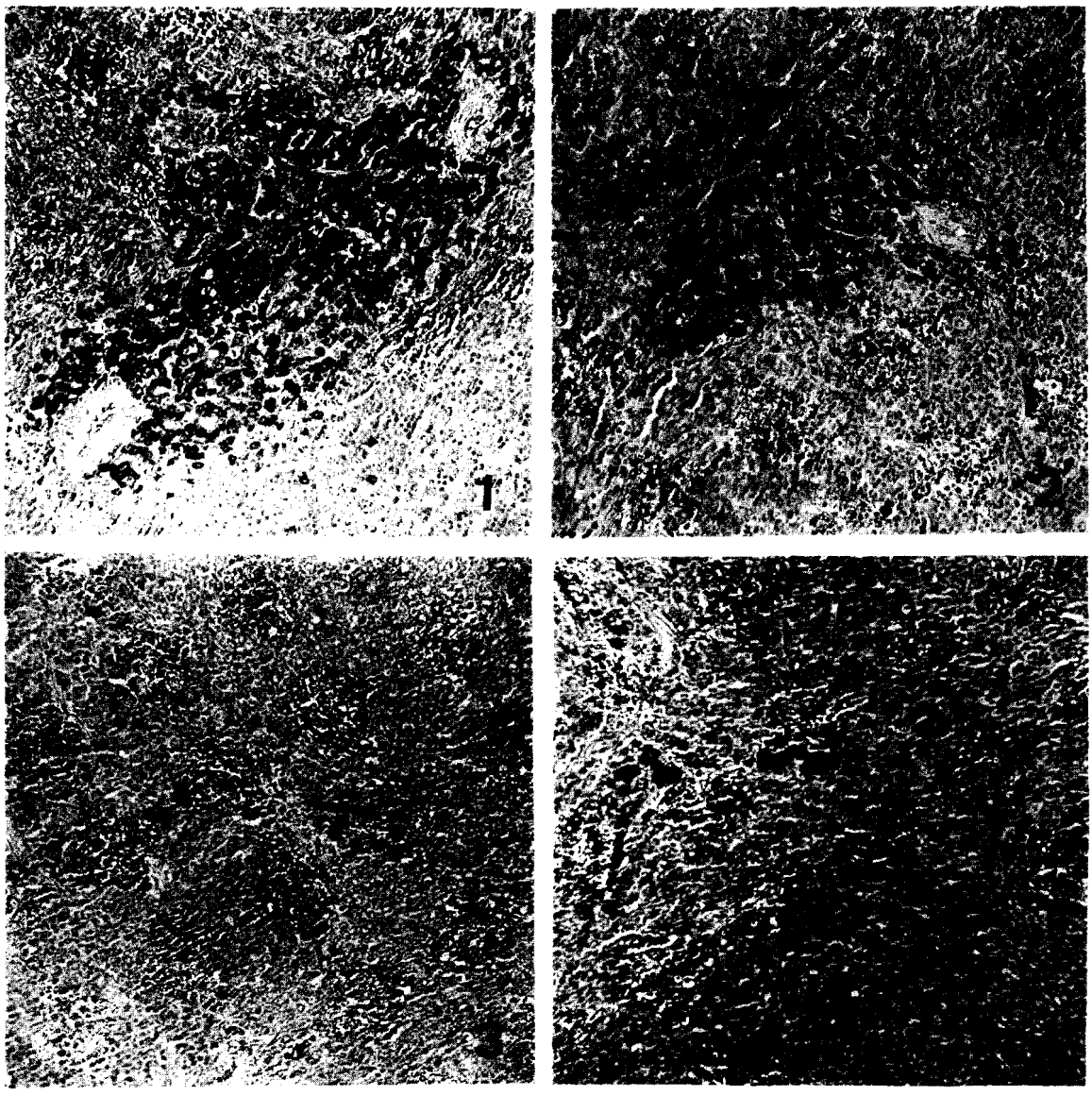
Group* (CY:mg/kg)	Body weight (g)**	Organ weight (g%)		
		Thymus	Spleen	Liver
A (0)	26.7 ± 1.7***	0.150 ± 0.022	0.336 ± 0.025	5.26 ± 0.17
B (0)	26.7 ± 1.6	0.158 ± 0.018	0.981 ± 0.042	5.80 ± 0.20
C (140)	27.0 ± 2.1	0.143 ± 0.013	1.100 ± 0.048	5.84 ± 0.29
D (210)	26.5 ± 1.3	0.161 ± 0.007	0.867 ± 0.148	5.54 ± 0.25
E (280)	25.5 ± 1.2	0.144 ± 0.042	0.492 ± 0.014	5.36 ± 0.15
F (280x2)	23.9 ± 1.5	0.097 ± 0.022	1.142 ± 0.096	4.94 ± 0.15

* Groups B to F were treated with swine serum.
** Initial body weight: 24.6 ± 0.8 g
*** Mean ± S.D.

Table 2. Antibody titer and incidence and severity of bile duct lesion

Group* (CY: mg/kg)	Antibody titer**		Bile duct lesion		
	IgG	IgM	Incidence	Severity	
				IHBD***	EHBD
A (0)	–	–	–	–	–
B (0)	1024	256	7/7	mild	moderate
C (140)	1024	128	7/7	mild	moderate
D (210)	512	128	7/7	mild	moderate
E (280)	8>	32	7/7	–	mild
F (280x2)	8>	8>	0/7	–	–

* Groups B to F were treated with swine serum.
** The reciprocal of the highest dilution of serum when its OD value was more than mean + 3 s.d. of negative serum by ELISA method.
*** IHBD: Intrahepatic bile duct, EHBD: Extrahepatic bile duct



Figs. 1 to **4** are the mouse spleen. Immunoperoxidase staining. × 200.

Fig. 1. A mass of IgG-positive lymphocytes in periarteriolar area (Group B).

Fig. 2. A small cluster of IgG-positive lymphocytes in periarteriolar area (Group D).

Fig. 3. A small number of IgG-positive lymphocytes in perifollicular area (Group E).

Fig. 4. IgG-positive lymphocytes in perifollicular area (Control group).

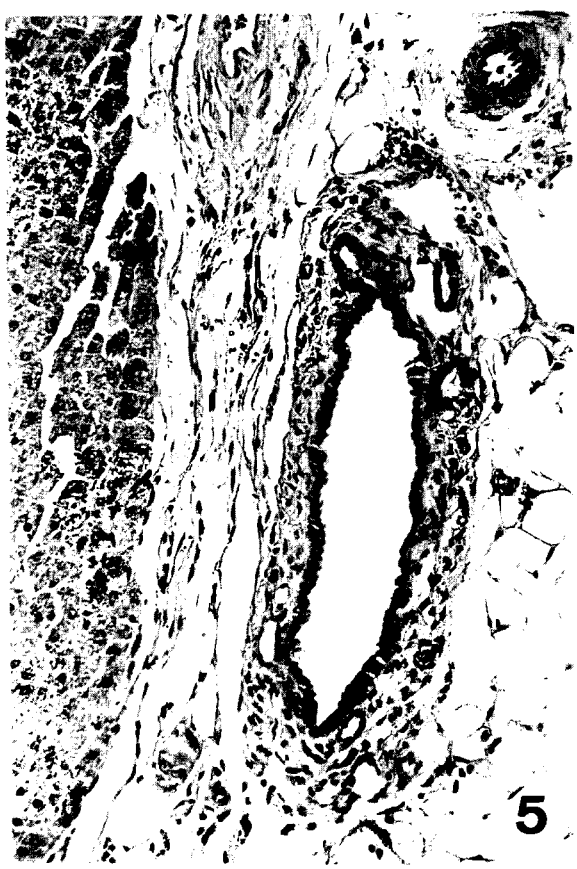
Figs. 5 to **8** are the mouse extrahepatic bile duct. Hematoxylin-eosin stain × 200.

Fig. 5. Normal picture (Control group).

Fig. 6. Hyperplasia of biliary epithelial cells, proliferation of mucous glands, and periductal infiltration of eosinophils with mild fibrosis (Group B).

Fig. 7. Similar bile duct lesion to that shown in Fig. 6 (Group C).

Fig. 8. Less severe bile duct lesion than that shown in Figs. 6 and 7 (Group E).



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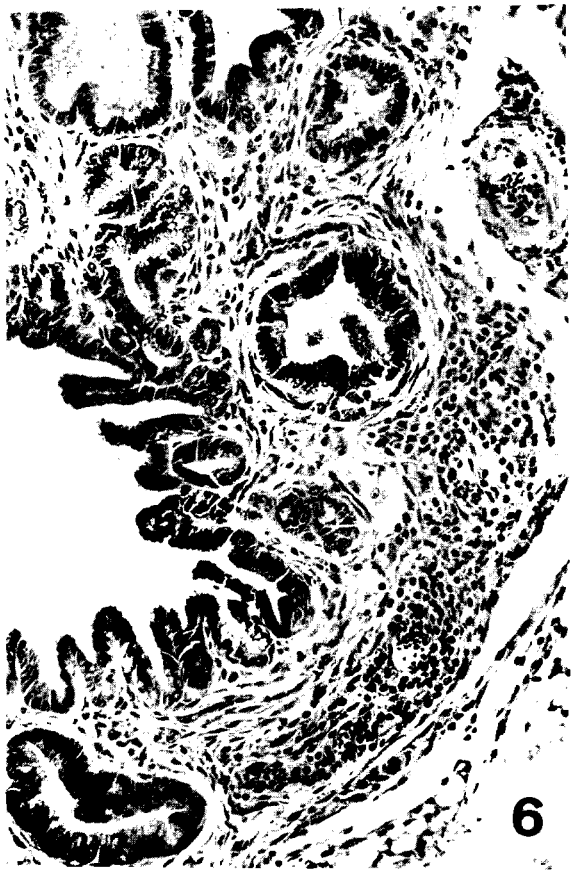

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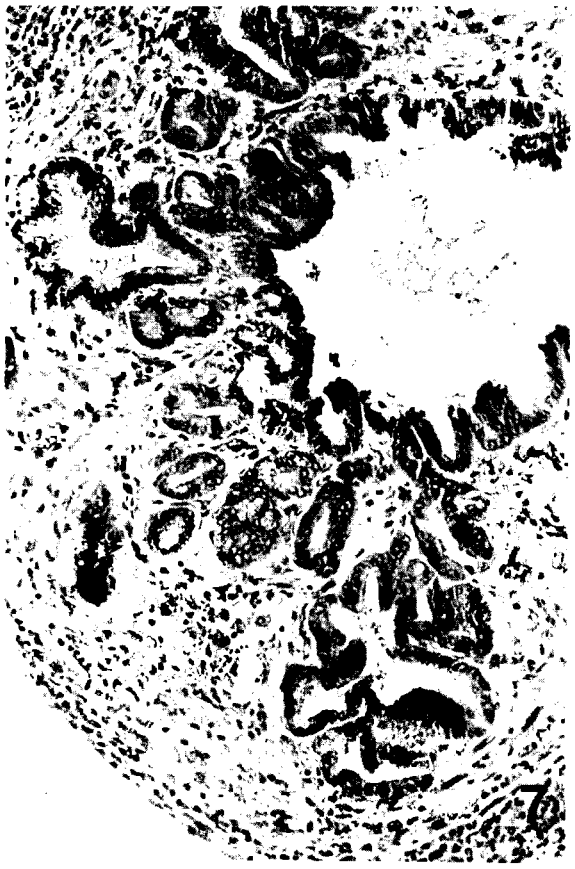

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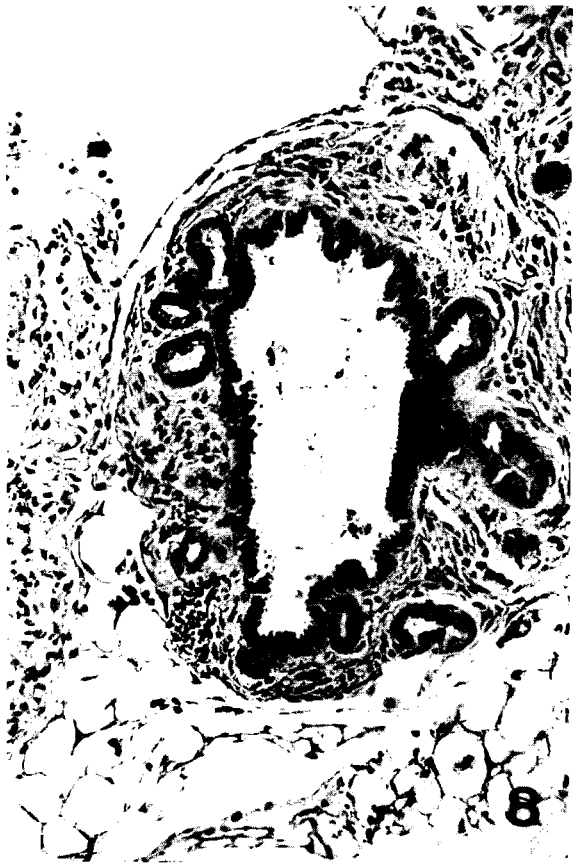

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Discussion

Mice given 280 mg x 2/kg b.w. of CY showed no production of anti-SS antibody and BDL, and the marked increase in their spleen weight was due to prominent extramedullary hematopoiesis probably in compensation for severe bone marrow cell damage by CY. Mice pretreated with a single dose of 140, 210 or 280 mg/kg b.w. of CY could respond to SS injection, and the severity of BDL was roughly proportional to the level of anti-SS antibody titer, which might reflect the population of immunoglobulin-producing splenic lymphocytes. These findings suggest at least in susceptible BALB/c mice that anti-SS antibody is important for the development of BDL and that heterologous serum protein might not produce extrahepatic bile duct enlargement unless immunoglobulin response occurs. It is true, but the level of anti-SS antibody titer necessary for the induction of minimal BDL in susceptible BALB/c mice was far lower than that expected from the previous paper (Imaoka et al., 1986; Doi et al., 1987). This seems to indicate that one or more unknown factors together with anti-SS antibody response are involved in the development of BDL.

In the CY-treated mice, except in mice given 280 × 2 mg/kg b.w. of CY, the weight and histology of the thymus were normal, and periarteriolar lymphocytes were less severely depressed than follicular lymphocytes even in the spleen of mice given 210 or 280 mg/kg b.w. of CY. However, modification of cellular immunity in the SS-induced BDL by CY should be investigated in the future, because a single injection of CY at the dose level used in the present study is said to have transient influence on not only humoral but also cellular immunity (Turk et al., 1972; Jokipii and Jokipii, 1973; Stockman et al., 1973; Kolb et al., 1977).

Glaser et al. (1987) reported that various heterologous sera including porcine serum produced a considerable and selective enlargement of extrahepatic bile ducts without inflammatory response and fibrosis under similar dosage and dosing schedule to ours. The SS-induced BDL in a series of our studies affected both the intrahepatic and extrahepatic bile ducts and accompanied more or less periductal eosinophilic infiltration and fibrosis (Doi et al., 1987; Itagaki et al., 1987, 1988). Although the reason is obscure, this difference in the pathological picture is very interesting.

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