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Foreign serum-induced bile duct lesion (BDL) in athymic BALB/c nude mice

K. Honjo* and K. Doi

Department of Pathology, Faculty of Agriculture, The University of Tokyo, Tokyo, Japan

*Present address: Department of Pathology, Division of Laboratory Medicine, University of Alabama, South Street, Birmingham, Alabama, USA

Summary. To investigate a role of cellular immunity in foreign serum-induced bile duct lesion (BDL) in mice, athymic BALB/c nude (nu/nu) mice were intraperitoneally injected with swine serum (SS) twice a week up to 8 weeks and were compared with euthymic BALB/c heterozygote (nu/+) and wild-type (+/+) mice treated with SS in the same way for 4 weeks. All immunized nu/+ and +/+ mice developed marked BDL, and their sera showed high anti-SS IgE and IgG1 antibody titers, whereas no immunized nu/nu mice developed lesions, and their sera showed no elevation of antibody titers. Next, nu/nu mice were reconstituted with splenocytes derived from nu/+ mice, and then were intraperitoneally injected with SS twice a week for 3 weeks. Most of the reconstituted nu/nu mice developed BDL, and their sera showed the elevation of anti-SS IgE and IgG antibody titers. These results suggest that cellular immunity may play a pivotal role in the pathogenesis of swine serum-induced BDL.

Key words: Cellular immunity, Foreign serum, Bile duct lesion, Nude mice, Splenocyte transfer

Introduction

Repeated intraperitoneal injections of foreign sera caused severe bile duct lesion (BDL) resulting in enlargement of bile duct in some strains of mice (Kitamura et al.,1985; Imaoka et al., 1986; Fallon-Friedlander et al., 1987). The highly susceptible strains of mice showed splenomegaly, enlargement of lymph nodes, eosinophilia, and hyperimmunoglobulinemia (especially IgG1 and IgE), and they developed BDL characterized by proliferation of mucous glands, hypertrophy and hyperplasia of biliary and glandular epithelia1 cells, infiltration of inflammatory cells and periductal fibrosis (Imaoka et al., 1986; Doi et al., 1987; Itagaki et al., 1987, 1988).

The precise pathogenesis of foreign serum-induced BDL is not clear. In this connection, Fallon-Friedlander et al. (1987) reported that purified human IgA produced bile duct proliferation identical to that induced with whole serum, and explained that heterologous IgA functioned as a specific extrahepatic bile duct growth factor. However histopathological findings suggest that the development of BDL may be somewhat attributed to immune responses (Doi et al., 1987). In addition, BDL was suppressed by cyclophosphamide treatment (Doi et al., 1990). Taking these facts into account, immune responses seem to play a role in the development of foreign serum-induced BDL.

To investigate whether immune responses are involved in BDL or not, athymic BALB/c nude mice were first treated with swine serum (SS) up to 8 weeks. Then, SS was injected into BALB/c nude mice which were reconstituted with splenocytes derived from heterozygote euthymic BALB/c mice.

Materials and methods

Animals

Male BALB/c-nu/nu, -nu/+ and -+/+ mice were used. They were obtained from Japan SLC Inc. (Shizuoka, Japan), and housed in filter-capped mouse cages under controlled conditions (temperature, 2322 °C; relative humidity, 55±5%), and given 5 mega-rad-irradiated CMF pellets (Oriental Yeast Co. Ltd., Tokyo) and autoclaved water ad libitum. For the evaluation of IgE titer by the passive cutaneous anaphylactic reaction (PCA) method, 20 8-week-old male Sprague-Dawley (SD) rats (Japan SLC Inc., Shizuoka, Japan) were used.

Foreign serum

Swine serum (SS) (Irvine Scientific, CA, USA) was used as foreign serum.

Offprint requests to: Prof. K. Doi, Department of Veterinary Pathology, Faculty of Agriculture, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan. Fax: +81-3-5841-8185. e-mail: akunio@mail.ecc.u-tokyo.ac.jp

Attempt to produce BDL in nu/nu mice by SS injection

Fifteen 5-week-old BALB/c-+/+, 15 5-week-old BALB/c-nu/+, and 29 4-week-old BALB/c-nu/nu mice were used. Nine +/+, 9 nu/+, and 8 nu/nu mice were intraperitoneally injected with 0.2 ml/head of sterile SS twice a week for 4 weeks. Another 9 nu/nu mice were injected with SS in the same way for 8 weeks to estimate the effect of prolonged treatment. The remaining mice given sterile saline in the same way served as controls.

Effect of adoptive transfer of splenocytes to nu/nu mice

Splenocytes were prepared from SS-primed or non-primed euthymic nu/+ mice. As primed mice, 4 5-week-old mice were injected with SS twice a week for 4 weeks as described above, and splenocytes were prepared 3 days after the last SS injection. Ten 7-week-old nu/+ naive mice were used as the donor of non-primed splenocytes. A total of 50 nu/nu mice were intravenously or intraperitoneally reconstituted with $5x10^6$ to $1x10^8$ splenocytes. Reconstituted nu/nu mice were intraperitoneally injected with SS twice a week for 3 weeks. In addition, 4 primed cell-transferred mice which were injected with sterile saline instead of SS served as controls.

Antibody titers

Anti-SS antibody titers were measured by ELISA method (IgG, IgM and IgA classes, and IgG subclasses) with polyclonal goat anti-mouse IgG, IgM, IgA (Organon Teknika Co., PA, USA), and IgG subclass (Funakoshi Co., Ltd., Tokyo, Japan) antibodies according to the method previously described (Doi et al., 1987). The results were described as the reciprocal of the highest dilution of serum when its optimal dose (OD) value was more than twice that of negative serum.

Anti-SS IgE titer was evaluated in SD rats by the PCA method (Watanabe and Ovary, 1977) with a little modification. To sensitize rats, 2.0 ml of sterile SS were intravenously injected via the tail vein. Immediately after, 1.0 ml of 0.5% Evans blue in PBS (pH 7.2) was additionally injected in the same way.

In the study of adoptive transfer to nu/nu mice, anti-SS IgG and IgE antibody titers were measured. The OD value of IgG antibody titer in serum samples was converted to percentage value using OD values in serially diluted standard positive serum.

Peripheral eosinophil counts

To investigate whether SS-treated nu/nu mice developed eosinophilia or not, orbital sinus bloods were collected from nu/nu and +/+ mice treated with SS (n=6 to 9) or saline (n=4 to 6) 40hr after the last injection. Eosinophils were stained with Hinkelman's solution (Huntsman et al., 1959) and counted.

Histopathology

All mice were killed by heart puncture under ether anesthesia 3 days after the last SS-injection. After measuring the outer diameter of common bile duct, the liver and common bile duct were fixed in 10% neutral-buffered formalin. Paraffin sections (4 μm) were stained with hematoxylin and eosin (HE) and subjected to the histopathological examination.

Statistical analysis

Values are expressed as mean±standard error (SE). Mann-Whitney U test and Spearmann rank correlation coefficient were used to analyze the data.

Results

Comparison of athymic nude mice with euthymic mice

As shown in Fig. 1, diameters of common bile ducts of athymic nu/nu mice were identical to those of saline-treated control nu/nu mice, while those of SS-treated euthymic +/+ and nu/+ mice were significantly larger than those of saline-treated controls. In euthymic mice, repeated SS-treatments induced severe BDL characterized by proliferation of mucous glands, hypertrophy and hyperplasia of biliary and glandular epithelial cells, infiltration of lymphoid cells and eosinophils, and periductal fibrosis (Fig. 2a).

SS-treated euthymic mice showed eosinophilia (Fig. 3) and elevation of anti-SS antibody titers, especially IgG1 and IgE titers (Table 1). On the other hand, no histopathological changes were observed in nu/nu mice even after 16 SS-treatments (Fig. 2b). Furthermore, nu/nu mice demonstrated no elevation of eosinophil counts (Fig. 3) or antibody titers (Table 1).

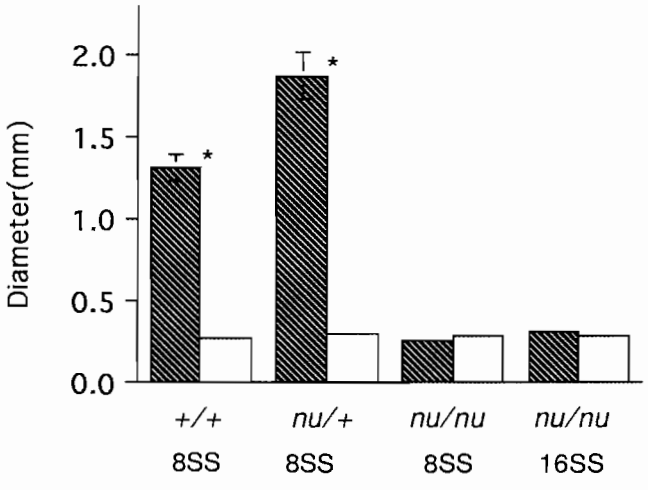

Fig. 1. Diameters of common bile ducts of nu/nu , nu/+ and +/+ mice injected with SS (square with oblique lines) or saline (square). Values are expressed as mean±SE. *: significantly different from control ($p<0.001$).

Effect of adoptive transfer to nude mice

When nu/nu mice were reconstituted with primed or non-primed splenocytes, most of these nu/nu mice revealed immune responses to SS and developed slight to severe BDL (Table 2). Histopathological characteristics of BDL were identical to those in euthymic mice (Fig. 4a). In general, nu/nu mice reconstituted with primed cells produced more severe BDL than those with non-primed cells, and 4 control mice did not develop BDL (Fig. 4b).

Sera from reconstituted nu/nu mice showed the elevation of anti-SS IgG and IgE antibody titers. Significant correlations between common bile duct diameters and IgG titers ($rs=0.73$, $p<0.01$) (Fig. 5a) and between common bile duct diameters and IgE titers ($rs=0.69$, $p<0.01$) (Fig. 5b) were obtained by Spearmann rank correlation coefficient.

Discussion

In this study, we elucidated that repeated intraperitoneal SS-injections did not induce BDL, elevation of antibody titers or peripheral blood eosinophilia in BALB/c nude mice. However nude mice reconstituted with functional splenocytes acquired the responsibility to immunization with SS and developed BDL comparable to those in euthymic mice. Taking into account that nude mice are athymic animals and virtually absent of functional T cells (Hunig, 1983), these results suggest that cellular immunity, especially T cell immunity, may be involved in the development of SS-induced BDL.

T cells play a vital role in regulating network between lymphoid cells and hematopoietic cells (Miyajima et al., 1988) and play a significant role in experimentally-induced immunological diseases (DeJoy et al., 1989; Mahi-Brown and Tung, 1989; Craighead et al., 1992; Takeda et al., 1996). Activated T cells secrete multipotential cytokines (Mosmann and Coffman, 1989; Kelso and Metcalf, 1990; Mosmann et al., 1991), and these cytokines affect a variety of inflammatory and hematopoietic cells and regulate immune responses. Based on the above-mentioned characteristics of immunological responses and histopathology of SS-induced BDL and the report of Tracey et al. (1988) showing that proliferation of bile ducts were induced by

Table 1. Anti-SS antibody titers in euthymic and athymic nude mice.

NUMBER OF	+/+	*nu/+*	*nu/nu*	
SS INJECTION	8	8	8	16
IgG	20480-40960	20480-40960	<5	<5
IgG1	NT	40960	NT	NT
IgG2a	NT	<10	NT	NT
IgG2b	NT	<10	NT	NT
IgG3	NT	<10	NT	NT
IgA	NT	<5-20	<5	<5
IgM	NT	80-160	<5	<5
IgE	80	80	<5	<5

NT: not tested.

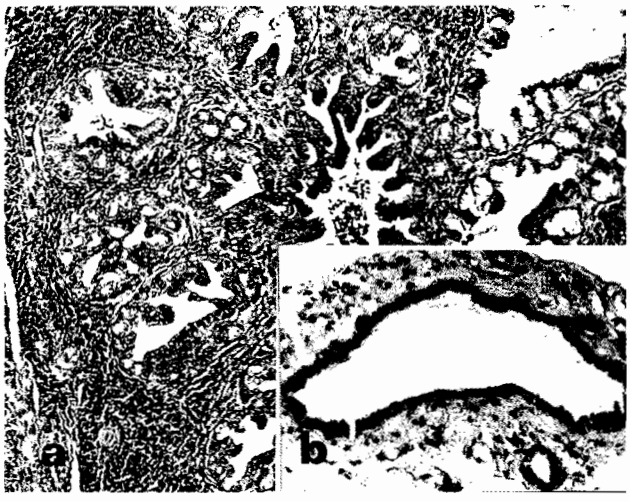

Fig. 2. Common bile ducts of +/+ mice after 8 SS-treatments (a) and of nu/nu mice after 16 SS-treatments (b). **a.** Proliferation of biliary epithelial cells and mucous glands is seen. HE, x 100. **b.** No clear changes are seen. HE, x 200

Table 2. Severity of BDL in reconstituted nu/nu mice.

	No. OF MICE	SEVERITY			
		none	slight	mild	severe
Primed					
$5x10^6$	10	0	2	6	2
$2x10^7$	10	1	1	3	5
Non-primed					
$5x10^6$	9	1	7	1	0
$2x10^7$	9	1	3	3	2
$1x10^8$	8	1	4	1	2

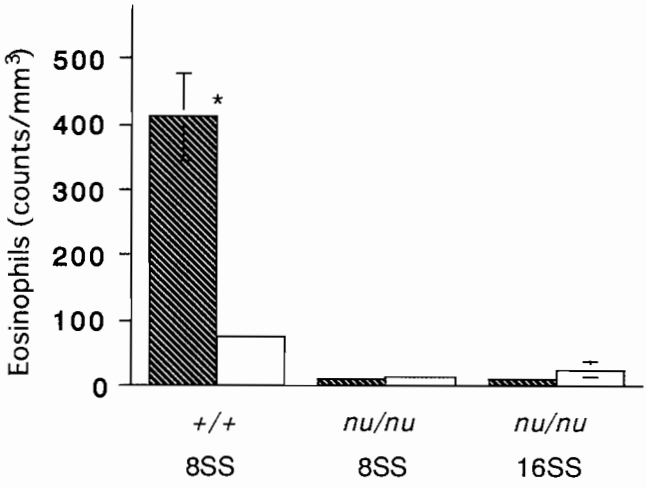

Fig. 3. Peripheral blood eosinophil counts of nu/nu and +/+ mice injected with SS (square with oblique lines) or saline (square). Values are expressed as mean±SE. *: significantly different from controls ($p<0.001$).

injections of TNF, T cells and T cell-derived cytokines seem to play an important role in the pathogenesis of SS-induced BDL. Further investigations will be needed to clarify the most functional T cell subpopulation(s) for the induction of BDL.

As mentioned above, Fallon-Friedlander et al. (1987) reported that purified human IgA produced bile duct proliferation identical to that induced with whole human serum, and explained that heterologous IgA itself or heterologous IgA-antibody complex functioned as a specific extrahepatic bile duct growth factor. If heterologous IgA itself functioned as an extrahepatic bile duct growth factor, SS could induce the BDL in nude mice. In fact, on the contrary, nude mice did not develop BDL. Therefore, it is difficult to assume that heterologous IgA directly functions as a bile duct growth factor. To clarify the other possibilty that IgA-antibody complex functions as an extrahepatic bile duct growth factor, a study on transfer of immune-complex to nude mice should be be done.

In general, nu/nu mice reconstituted with primed splenocytes developed more severe lesions than those with non-primed splenocytes did. Possibly, primed cells may respond more rapidly and strongly to SS-sensitization than non-primed cells, and therefore nu/nu mice reconstituted with primed cells may develop more

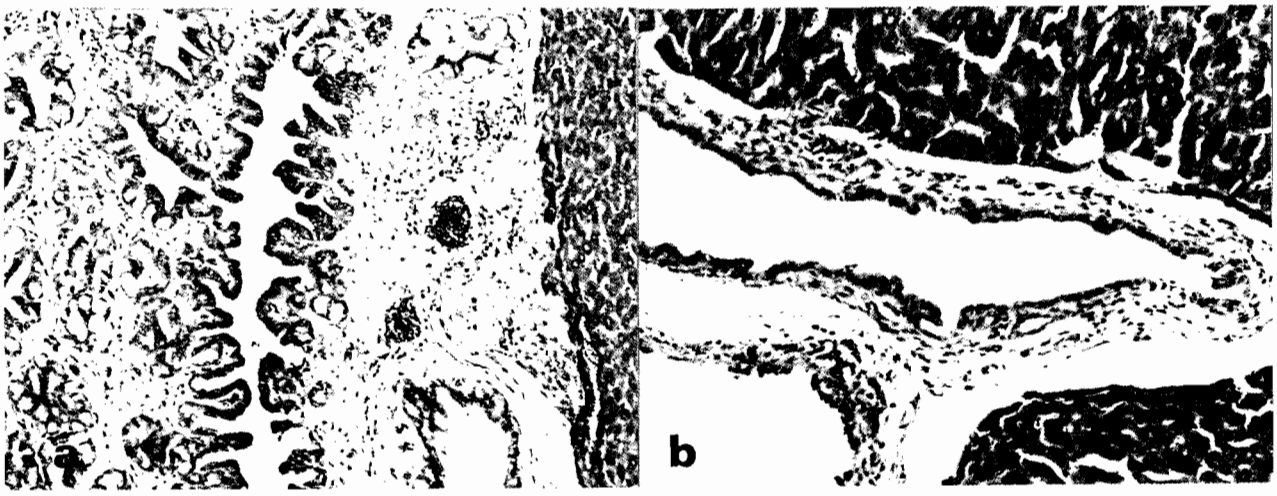

Fig. 4. Extrahepatic bile ducts of a nu/nu mouse reconstituted with primed splenocytes (a) and of a control nu/nu mouse (b). **a.** Proliferation of biliary epithelial cells and mucous glands is seen. H-E, x 100. **b.** No prominent changes are seen. HE, x 200

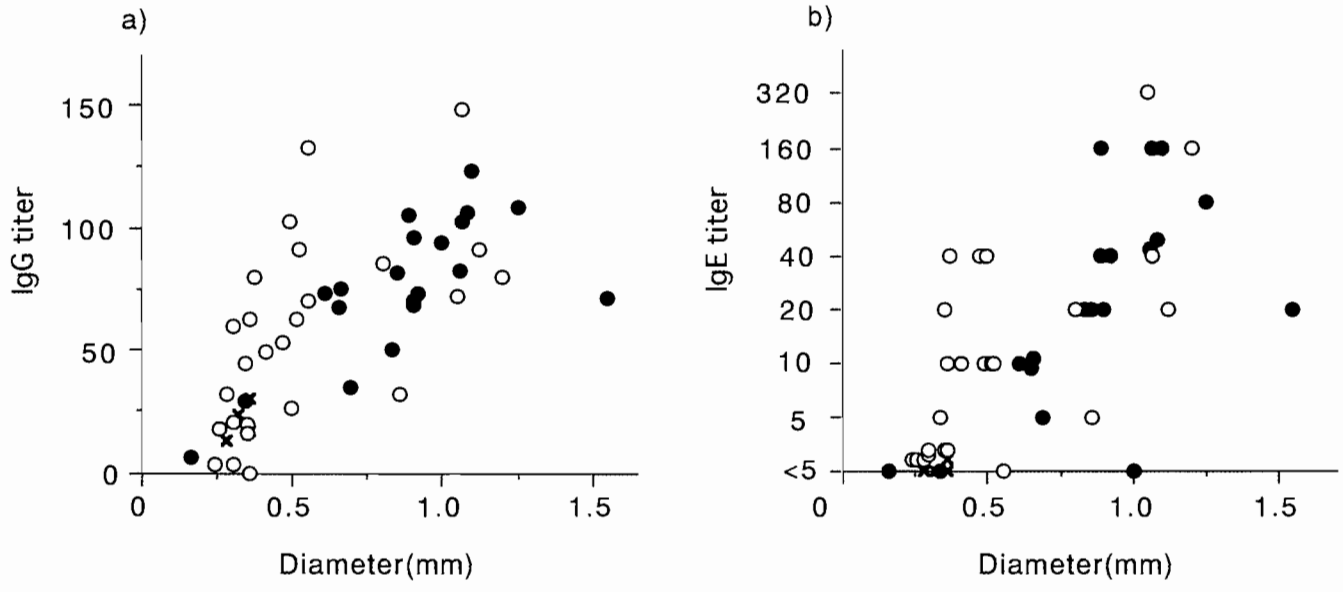

Fig. 5. Correlations between diameters of common bile ducts and IgG **(a)** and IgE antibody titers **(b)** in nu/nu mice reconstituted with primed (black circle) or non-primed spleen cells (circle). x: values of control mice.

severe lesion.

The enlargement of common bile ducts is considered to be attributed to fibrogenic response and cellular reactions such as hypertrophy and hyperplasia of biliary and glandular epithelial cells, hyperplasia of mucous glands, periductal fibrosis, and infiltration of inflammatory cells, and therefore the diameter of common bile ducts seems to reflect the magnitude of BDL. Judging from the significant correlations between common bile duct diameter and anti-SS IgG and IgE antibody titers in reconstituted nu/nu mice, it is reasonable to consider that anti-SS antibodies may also be involved in the development of BDL.

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