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Transgenic mice expressing osteopontin in hepatocytes as a model of autoimmune hepatitis☆

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Abstract

Osteopontin, a crucial factor for Th1 immune response, is expressed in stellate cells and macrophages activated in injured liver. To clarify the role of osteopontin in inflammatory changes in the liver, we attempted to establish transgenic mice expressing osteopontin in hepatocytes. Mouse osteopontin cDNA, cloned from concanavalin-A-stimulated spleen cells in C57BL/6 mice, was constructed into the vector containing serum amyloid-P component promoter. This construction was microinjected into fertilized eggs of C57BL/6 mice, and 4 lines of the transgenic mice were obtained. Western blotting and immunohistochemistry revealed that osteopontin was expressed in hepatocytes, but not in non-parenchymal cells, in the transgenic mice. The mean osteopontin concentrations in the liver and plasma in the mice were 13 and 2.6 times higher than those in negative littermates. Antinuclear antibody was positive in the plasma in 50% of the transgenic mice. In the transgenic mice later than 12 weeks of age, mononuclear cell infiltration in the liver developed, and these cells were positive for CD8 and HLA-DR. Plasma ALT activity was increased with focal necrosis in hepatic lobules in the transgenic mice later than 24 weeks of age. The transgenic mice expressing osteopontin in hepatocytes may be useful as a model of autoimmune hepatitis.
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Keywords: Autoimmune hepatitis; Cytotoxic T lymphocytes; Osteopontin; Transgenic mouse

Chronic hepatitis develops as a chronic inflammatory reaction in the liver of hepatitis viral infection and autoimmunity against hepatocytes, and often progresses to liver cirrhosis. Hepatocyte necrosis and apoptosis are common features of chronic hepatitis. It is widely accepted that such injuries occur through the immune reaction mediated by cyotoxic T lymphocytes (CTLs) [1]. Although interferon (IFN)-γ and tumor necrosis factor (TNF)-α are shown to be involved both in CTL activation and hepatocyte injuries [2,3], the cytokines responsible for initiation of Th1 immune reaction in chronic hepatitis are to be elucidated.

Osteopontin is a secreted glycoprotein of about 41,500 Da that can bind to hydroxyapatite and calcium [4,5]. This protein is expressed mainly in the bone and kidney, and is shown to contribute physiologically to extracellular matrix formation and calcium deposition in these organs [5]. It has long been believed that osteopontin is not expressed in the liver of normal rats and mice [5]. We demonstrated that both mRNA and protein of osteopontin were abundantly expressed in Kupffer cells, macrophages, and stellate cells activated in the liver of rats given carbon tetrachloride (CCl_4) or heat-killed *Propionibacterium acnes* (*P. acnes*) [6,7]. Osteopontin can bind to αvβ3 integrin on monocytes and macrophages through the RGD motif [8], and promote migration of these cells both in vitro and in vivo [8,9]. Also, osteopontin is reported to act as a cytokine essential for initiation of Th1 immune response in mice [10]. Thus, it is strongly suggested that osteopontin may play a crucial role in the development of chronic liver injuries.

We made transgenic mice expressing osteopontin exclusively in hepatocytes using the vector containing

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human serum amyloid P component promoter, and found that focal necrosis with abundant infiltration of CTLs occurred spontaneously in the liver. The present paper reports immunological and pathological features of these mice, and their usefulness as a model of autoimmune hepatitis.

Materials and methods

Construction of osteopontin transgene. Mouse osteopontin cDNA (0.9 kb) was isolated by RT-polymerase chain reaction (PCR) using total RNA prepared from concanavalin-A-activated spleen cells of C57BL/6 mouse (Japan SLC, Hamamatsu, Japan). *Hin*dIII site in the open reading frame of mouse osteopontin cDNA was abolished by silent mutation using a standard PCR method. The mutated osteopontin cDNA was then cloned into the *Eco*RI site of pLG1-SAP vector [11] that contains human serum amyloid P component (SAP) promoter and rabbit β-globin gene (Fig. 1). The resultant plasmid was digested with *Hin*dIII and *Xho*I, and the 2.7-kb fragment of SAP-osteopontin gene was isolated and used for microinjection.

Generation and screening of osteopontin transgenic mice. Fertilized eggs were obtained from C57BL/6 mice, and several hundred molecules of the SAP-osteopontin fragment were injected into the pronucleus of the fertilized eggs, as previously described [12]. The resultant eggs were transplanted into the oviducts of foster mothers. Genomic DNA was extracted from the tail of newborn mice at 4 weeks of age and subjected to polymerase chain reaction (PCR) assay to detect the transgene. Sense and antisense primers were synthesized based on cDNA sequences of 5′ non-coding region of rabbit β-globin gene and the osteopontin gene, respectively; 5′-TGC TGT CTC ATC ATT TTG GC-3′ for sense primer and 5′-GCA GGC TGT ATA GCT TCT CCT-3′ for antisense primer (Fig. 1).

All mice were maintained on a commercial pelleted diet and water ad libitum in a room at 22 ± 2 °C under normal laboratory lighting conditions. All animal protocols conformed to the Guide for Care and Use of Laboratory Animals by the National Academy of Sciences.

Determination of alanine aminotransferase (ALT) activity, osteopontin concentration, and antinuclear antibody in plasma. Blood was obtained from mice under anesthesia with ether using a syringe containing a 1:10 volume of 3.8% sodium citrate for preparation of plasma.

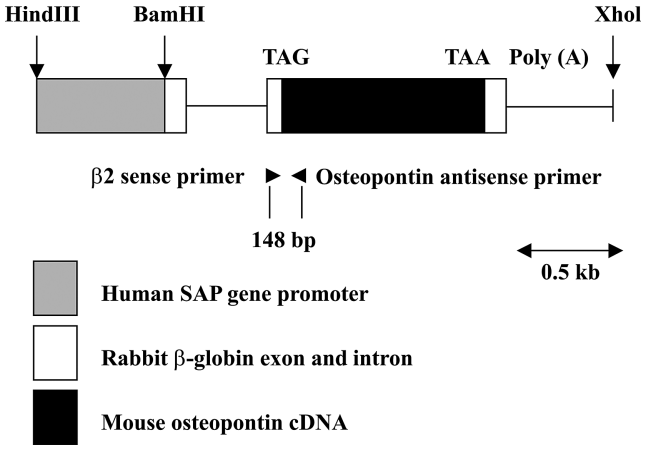

Fig. 1. Structure of the human serum amyloid P component (SAP)-osteopontin transgene. Arrows indicate the sites of restriction endonucleases. Arrowheads mean primers used in polymerase chain reaction (PCR) to detect the transgene.

Plasma ALT activity and osteopontin concentration were determined using commercial kits (Reflotron system for ALT; Iatron Laboratories, Tokyo, Japan, and an ELISA kit for mouse osteopontin; Immuno-Biological Laboratories, Fujioka, Japan).

Antinuclear antibody was evaluated in plasma by a two-step indirect fluorescent procedure using HepG2 slides (SRL, Tokyo, Japan) and FITC-conjugated goat polyclonal antibody against mouse IgG and IgM (Immunotech, Marseille France). Antinuclear antibody was determined as positive, when fluorescent signals were found on the nuclei following the reaction with the plasma diluted 40 times with phosphate-buffered saline (PBS).

Isolation of liver cells. The liver was perfused through the portal vein with 0.05% collagenase (type I: Worthington Biochemical, Freefold, NJ) in Hanks' balanced salt solution (HBSS: Nissui Pharmaceutical, Tokyo, Japan) at a flow rate of 1 ml/min at 37 °C for 15 min and excised. Then, the liver was minced and filtered through a nylon mesh. The resultant suspension was centrifuged two times at 125*g* for 5 min in Ca^{2+}- and Ma^{2+}-free HBSS (Gibco Laboratories, Life Technologies, Grand Island, NY) at 4 °C, followed by centrifugation two times at 70*g* in the similar solutions, and the cells in the pellets were collected as hepatocyte-rich fraction. In another experiment, the suspension was centrifuged at 1400*g* for 15 min in 18% metrizamide (Sigma Chemical, St. Louis, MO) in Ca^{2+}- and Ma^{2+}-free HBSS at room temperature. The cells in the top layer of the solution were collected as non-parenchymal cell-rich fraction.

The viability of the cells in hepatocyte- and non-parenchymal cell-rich fractions immediately after isolation was more than 95% by trypan blue exclusion test, and the purity of the cells assessed on light microscopy was greater than 99%.

Western blot analysis and ELISA assay for osteopontin in various organs and liver cells. The liver, kidney, lung, spleen, pancreas, stomach, intestine, testis or ovary, submandibular gland, thymus, heart, and brain were obtained immediately after blood collection. Excised organs and the cells in hepatocyte- and non-parenchymal cell-rich fractions were homogenized at 4 °C in a solution of 0.5% sodium dodecyl sulfate (SDS) and 60 mM of 2-amino-2-hydroxymethyl-1,3-propanediol–HCl (pH 6.8). The resultant suspension was boiled for 5 min and disrupted by sonication. The samples containing 5 μg of protein were subjected to electrophoresis in SDS–polyacrylamide gel (12% acrylamide). Proteins in the gel were transferred onto nitrocellulose paper (Hybond-C; Amersham International, Buckinghamshire, England) using Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell (Bio-Rad, Richmond, CA). The paper was soaked in a blocking agent made from skim milk (BLOCK ACE; Snow Brand Milk Product, Sapporo, Japan) and exposed to polyclonal antibody against mouse osteopontin (IBL) as a primary antibody and alkaline phosphatase-conjugated goat antibody against rabbit IgG (ICN Pharmaceuticals, Aurora, OH) as a secondary antibody. Products of the reaction were visualized with 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium (BCIP/NBT: Gibco Laboratories) as a developing agent.

In another experiment, excised livers and kidneys were homogenized at 4 °C in a solution containing 0.3 mM PMSF, 3% Tween 20, 0.15 M NaCl, and 10 mM Tris–HCl (pH 7.4), and the supernatants were collected by centrifugation. They were subjected to ELISA for osteopontin similarly as was done in plasma.

Histological examination of organs. Excised organs were fixed in 20% formalin neutral buffer solution (Muto Pure Chemicals, Tokyo, Japan) and embedded in paraffin. Four-micrometer sections of each organ block were deparaffinized, and observed on light microscopy after staining with hematoxylin and eosin.

Immunohistochemical examination of the liver. For immunostaining of osteopontin, deparaffinized sections of liver blocks similarly made as was in histological examination were used. For immunostaining of CD4, CD8, and HLA-DR, blocks of 5 mm^3 cut off with a blade from the excised liver were put into OCT compound (Sakura Finetechnical, Tokyo, Japan) and rapidly frozen in dry ice–acetone. Four-micrometer cryostat sections were made from the frozen liver blocks. In both

staining, endogenous peroxidase activity and non-specific binding site were blocked in PBS containing 0.3% H_2O_2 and in whole rabbit serum, respectively. Immunohistological staining was performed by avidin–biotin–peroxidase complex (ABC) method [13] with Vectastain ABC kit (for rabbit or rat IgG: Vector Laboratories, Burlingame, CA), using rabbit polyclonal antibody against mouse osteopontin (Immuno-Biological Laboratories) and rat monoclonal antibodies against CD4 (Oxford Biotechnology, Portsmouth, NH) CD8 (Oxford Biotechnology), and HLA-DR (AbCam, Cambridge, UK). Non-immune rabbit or rat IgG at the same concentration was used as the controls of primary antibodies. The reaction product was developed by incubation in solution of 0.05% diaminobenzidine tetrahydrochloride, pH 7.2, containing 0.01% H_2O_2 (Vector Laboratories). Counterstaining was done in 4% methyl green solution.

Results

Transgenic mouse lines expressing osteopontin

Among 52 mice brought up after the transplantation, 6 mice (3 males and 3 females) carried the transgene. These 6 mice were mated with C57BL/6 mice at 8 weeks of age, and the transgene was transmitted to the offspring of 4 lines; the SAP-osteopontin 17, 35, 43, and 73 lines. The transgenic mice were mated each other in the same lines, and the offspring carrying the transgene derived from the SAP-osteopontin 17, 43, and 73 lines were used in the experiments. The offspring from the SAP-osteopontin 35 line was deleted from the analysis, because breeding efficacy was minimal. The mice of the SAP-osteopontin 17, 43, and 73 lines were transferred to specific pathogen-free conditions by in vitro fertilization. All mice were alive until 1 year of age.

Osteopontin expression in various organs and liver cells

Western blot analysis revealed that osteopontin protein was expressed slightly in the kidney and lung, but not in the liver of negative littermates of the transgenic mice as well as in C57BL/6 mice (data not shown). However, marked expression of the protein was found in the liver of the transgenic mice regardless of the lines (Fig. 2A). In the transgenic mice, the protein was expressed slightly in the kidney and lung, but minimal in other organs such as brain, submandibular gland, thymus, heart, spleen, pancreas, stomach, intestine, and testis.

As shown in Fig. 2B, osteopontin protein was expressed in hepatocytes isolated from the transgenic mice. In contrast, the expression was minimal in isolated non-parenchymal cells. Also, osteopontin was stained diffusely in the cytoplasm of hepatocytes throughout the hepatic lobules in the transgenic mice (Fig. 3A). Such stains were absent in sinusoidal cells in the transgenic mice (Fig. 3A) and in hepatocytes of the negative littermates (Fig. 3B).

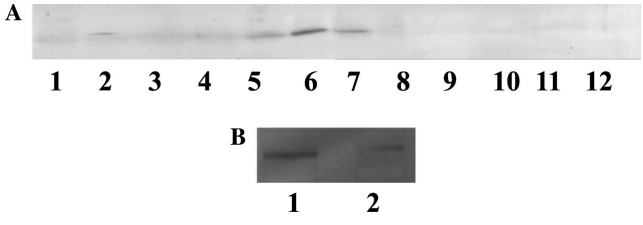

Fig. 2. Western blot analysis of osteopontin in a male transgenic mouse at 12 weeks of age of the SAP-osteopontin 17 line. (A) Osteopontin expressions in various organs. Lanes, 1: brain, 2: submandibular gland, 3: thymus, 4: heart, 5: lung, 6: liver, 7: kidney, 8: spleen, 9: pancreas, 10: stomach, 11: intestine, and 12: testis. Osteopontin is expressed markedly in the liver and slightly in the lung and kidney. (B) Osteopontin expressions in isolated liver cells. Lane 1: hepatocytes, lane 2: non-parenchymal cells. Osteopontin is expressed in hepatocytes, but not in non-parenchymal cells.

Osteopontin concentrations in the liver and plasma and ALT activity and antinuclear antibody in plasma

As shown in Fig. 4, osteopontin protein was detectable in the liver of the negative littermates at 12 weeks of age. The concentration (ng/g liver: mean ± SD) was 85 ± 18, which was significantly smaller than that in the kidney (131 ± 47, $p < 0.05$ by Student's t test). In contrast, the concentration was markedly increased in the liver of the transgenic mice (1139 ± 2419; ranging from 34 to 15,600) compared to that in the negative

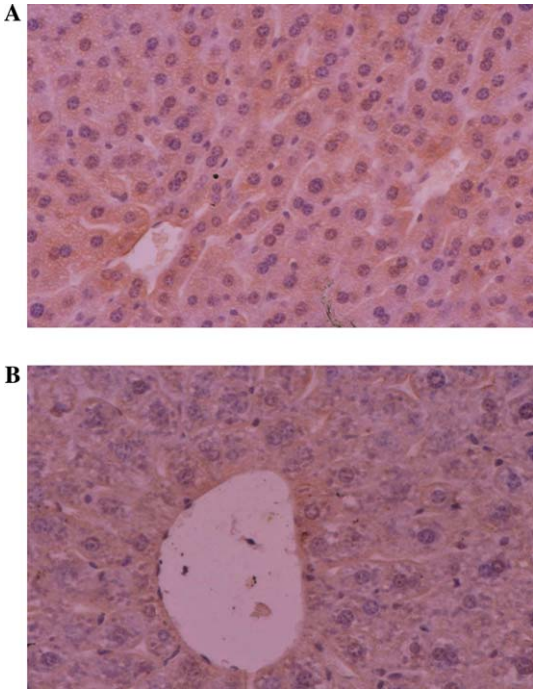

Fig. 3. Immunostaining of osteopontin in the liver in a female transgenic mouse and a female negative littermate at 12 weeks of age of the SAP-osteopontin 17 line. (A) Transgenic mouse, (B) negative littermate. Osteopontin is stained diffusely in the cytoplasm of hepatocytes throughout the hepatic lobules in the transgenic mouse. Such stains are absent in the liver in the negative littermate.

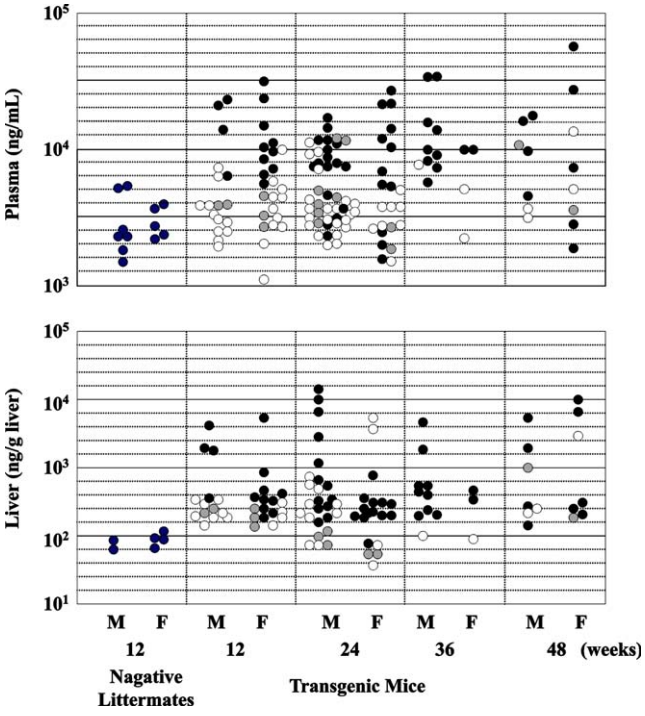

Fig. 4. Osteopontin concentrations in the liver and plasma in the transgenic mice. M and F mean male and female, respectively. Mice of the SAP-osteopontin indicate 17 line (●), 43 line (◍), and 73 line (○).

littermates ($p < 0.01$ by Mann–Whitney U test). The concentration was especially high in the liver of the mice of the SAP-osteopontin 17 line (1607 ± 2976). There was no difference in osteopontin concentrations in the liver between male and female mice of the 17 and 43 lines, while the concentration was higher in female mice (1333 ± 2022) than in male mice of the 73 line (262 ± 160, $p < 0.05$ by t test). The concentration did not differ in the transgenic mice depending on their age.

Plasma osteopontin concentration (ng/ml; mean ± SD) was increased in the transgenic mice (7890 ± 7752; ranging from 1150 to 55,000), being significantly higher than that in the negative littermates (3001 ± 1263; from 1525 to 5375, $p < 0.01$ by U test) (Fig. 4). The concentration was especially increased in the mice of the 17 line ($12{,}045 \pm 8581$), which was higher than that in the mice of the 43 and 73 lines (5121 ± 3361 and 4203 ± 2492, respectively; $p < 0.01$ by U test). The concentration did not differ between male and female mice of each line, but was increased with aging (6838 ± 6313, 6681 ± 5566, $12{,}313 \pm 9668$, and $12{,}114 \pm 13{,}788$ for 12, 24, 36, and 48 weeks, respectively; $p < 0.01$ by one-way analysis of ANOVA).

As shown in Fig. 5, plasma ALT activity (IU/L; mean ± SD) in the transgenic mice at 12 weeks of age (25 ± 11) was not different from that in the negative littermates (25 ± 6). However, the mice showing plasma ALT activity higher than 100 IU/L developed at 24 weeks of age (51 ± 60), and the levels increased

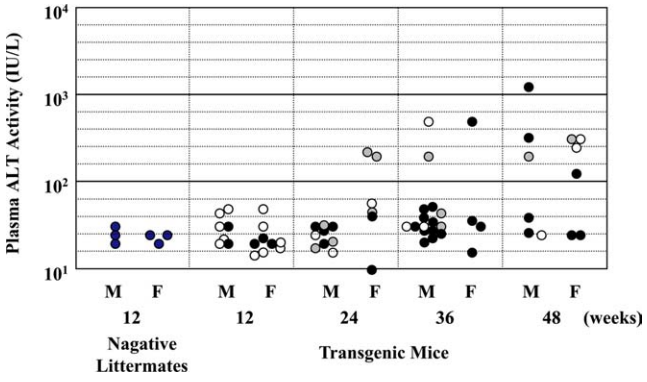

Fig. 5. Plasma alanine aminotransferase (ALT) activity in the transgenic mice M and F mean male and female, respectively. Mice of the SAP-osteopontin indicate 17 line (●), 43 line (◍), and 73 line (○).

depending on their ages (88 ± 150 and 234 ± 306 in the mice at 36 and 48 weeks of age, respectively; $p < 0.01$ by one way analysis of ANOVA). There were no differences in plasma ALT activity between male and female transgenic mice, and the relation was not found between plasma ALT activities and hepatic osteopontin concentrations in these mice.

Antinuclear antibody was evaluated in the plasma of 20 transgenic mice (3 males and 3 females of each of the SAP-osteopontin 43 and 73 lines, and 4 males and 4 females of the 17 line) and 6 negative littermates randomly selected. The antibody was negative in 6 negative littermates, but positive in 50% of the transgenic mice (4 males and 6 females; 4, 3, and 3 mice of the 17, 43, and 73 lines, respectively). Plasma osteopontin concentration in the transgenic mice with positive antinuclear antibody was $18{,}508 \pm 20{,}425$, which was not different from the concentration in the mice with negative antinuclear antibody ($27{,}641 \pm 28{,}661$).

Histological and immunohistochemical findings in the liver

Histological features of the liver in the transgenic mice were almost equivalent among three lines. Also, there was no difference between male and female transgenic mice.

No abnormal findings were present in the liver of the transgenic mice until 8 weeks of age. However, slight infiltration of mononuclear cells developed in the periportal areas in the transgenic mice at 12 weeks of age (Fig. 6A). In these mice, focal necrotic lesions with mononuclear cell infiltration were found in the midzonal areas of the hepatic lobules (Fig. 6B). Immunohistochmical examination revealed that these infiltrating cells were negative for CD4, but positive for CD8 and HLA-DR (Figs. 7A–C). Also, HLA-DR was markedly stained on sinusoidal cells throughout the lobules (Fig. 7D).

Infiltration of mononuclear cells became more prominent in the liver in the transgenic mice with aging. As shown in Fig. 6C, marked infiltration of the cells

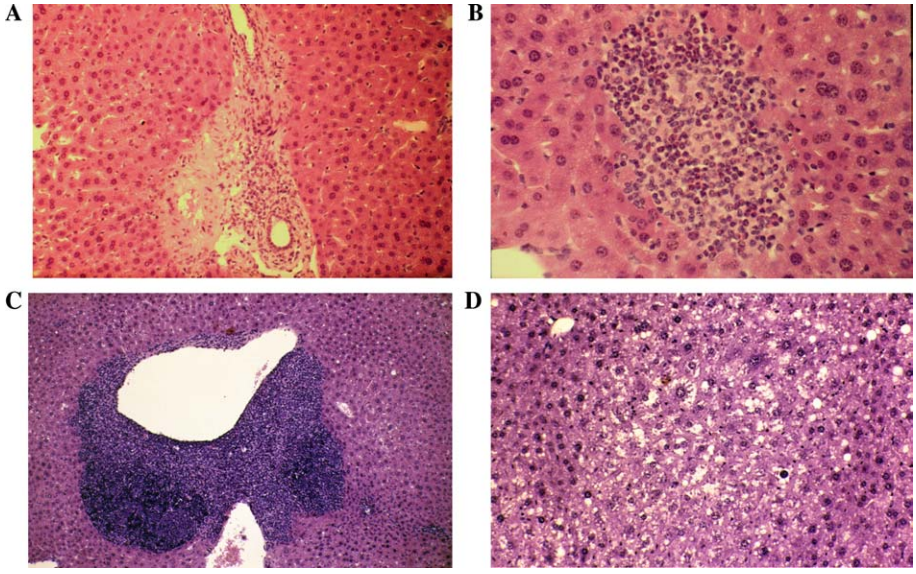

Fig. 6. Histological features of the liver in the transgenic mice (hematoxylin and eosin staining). (A) A male transgenic mouse at 12 weeks of age of the SAP-osteopontin 73 line. Mononuclear cell infiltration is seen in the periportal area of the hepatic lobules (original magnification at 200×). (B) A male transgenic mouse at 12 weeks of age of the SAP-osteopontin 73 line. Focal necrosis with mononuclear cell infiltration is found in the midzonal area of the hepatic lobules (400×). (C) A male transgenic mouse at 48 weeks of age of the SAP-osteopontin 43 line. Massive mononuclear cell infiltration is seen in the midzonal and pericentral areas of the hepatic lobules (100×). (D) A male transgenic mouse at 48 weeks of age of the SAP-osteopontin 17 line. Vacuolar degeneration in the cytoplasm is apparent in hepatocytes in the midzonal area of the hepatic lobules (200×).

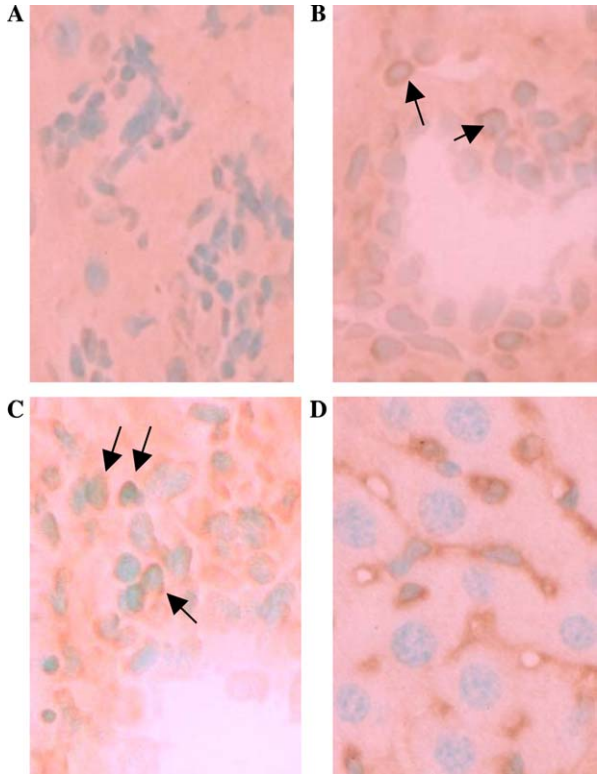

Fig. 7. Immunostaining of CD4, CD8, and HLA-DA in the liver of a female transgenic mouse at 12 weeks of age of the SAP-osteopontin 17 line (counterstained with methyl green, original magnifications at 400×). Staining of CD4 (A) is absent on infiltrating mononuclear cells in the periportal areas, but CD8 (B) and HLA-DR (C) are stained on the surface of infiltrating cells. HLA-DR staining is also positive on sinusoidal cells (D) throughout the hepatic lobules.

occupying the midzonal and centrizonal areas was seen in the liver of the transgenic mice at 48 weeks of age. In these mice, hepatocytes showed vacuolar degeneration in the cytoplasm especially in the midzonal areas of the hepatic lobules (Fig. 6D). However, the size of focal necrotic areas did not differ between the transgenic mice of 12 and 48 weeks of age.

Discussion

In the present study, four lines of osteopontin transgenic mice were obtained. We used a pLG1-SAP vector [11] that contains the human SAP promoter consisting of a motif putatively recognized by hepatocyte nuclear factor (HNF)-1 [14] and the exon and intron of rabbit β-globin gene, because osteopontin may be expressed exclusively in hepatocytes in such transgenic mice. In fact, Western blot analysis using isolated liver cells and immunohistochemical examination revealed that osteopontin protein was expressed abundantly in hepatocytes, but not in non-parenchymal cells (Figs. 1B and 3A). Moreover, osteopontin expressions in other organs than the liver were equivalent between the transgenic mice and negative littermates on Western blotting (Fig. 1A), suggesting that this transgenic mice may be useful as a tool for evaluating the role of liver-derived osteopontin in the development of chronic liver diseases in mice.

The amount of osteopontin protein produced by hepatocytes in the transgenic mice was assessed by an ELISA both in the liver and plasma, since osteopontin is

a secretory protein as well as an extracellular matrix protein [4,5]. As shown in Fig. 3, osteopontin concentrations in the liver and plasma varied widely among the transgenic mice. It is noteworthy, however, that these concentrations were increased either in the liver or plasma in most of the transgenic mice; there existed only seven mice (5.0%) in which both concentrations in the liver and plasma did not exceed the maximal levels in the negative littermates (114 ng/g liver for the liver and 5375 ng/ml for plasma). As a result, the mean concentrations in the liver and plasma were about 13 and 2.6 times greater, respectively, in the transgenic mice compared to those in the negative littermates.

Histological examination revealed that mononuclear cell infiltration occurred spontaneously in the liver of the transgenic mice. The infiltration was seen in the periportal areas in these mice at 12 weeks of age and became more prominent with aging; massive mononuclear cell infiltration occupying the midzonal and centrilobular areas was found in the transgenic mice at 48 weeks of age (Fig. 6C). These infiltrating cells were identified as CTLs from the positivity for CD8 and HLA-DR on immunohistochemistry. Also, HLA-DR was stained along the hepatic sinusoids throughout the lobules, suggesting that Kupffer cells were activated in the transgenic mice. Thus, it would be reasonable to assume that osteopontin production by the liver is sufficient to initiate Th1 immune response and promote activation of Kupffer cells and migration of CTL from the blood stream into the liver.

Vacuolar degeneration and focal necrosis of hepatocytes developed in the hepatic lobules with CTL infiltration in the transgenic mice. Plasma ALT activity was increased up to 1152 IU/L, and plasma antinuclear antibody was positive in 50% of these mice. Such findings were in line with clinical characteristics of autoimmune hepatitis in human. It is well known that autoimmune hepatitis occurs predominantly in female patients. However, histological features in the liver and the positive ratio of antinuclear antibody did not differ between our male and female transgenic mice. Thus, it seems likely that osteopontin production by the liver can provoke hepatocyte injury through CTL infiltration even in the male transgenic mice. The target molecules on hepatocytes recognized by CTL should be examined in future. Also, plasma osteopontin concentration did not differ between the mice with and without antinuclear antibody. Moreover, there existed no relation between hepatic osteopontin concentrations and plasma ALT levels in the transgenic mice. The mechanisms of osteopontin involved in the formation of antinuclear antibody and development of liver necrosis must be elucidated.

Various immune disorders are found in patients with autoimmune hepatitis. B lymphocyte activation and plasma cell infiltration are observed in the liver, and hyper-γ-globulinemia develops almost in all of the patients [15]. In the present experiments, however, we could not measure plasma γ-globulin concentration in the transgenic mice, since ELISA system adaptable for mice plasma was not yet established in our laboratory. Immunological features of the transgenic mice should be studied especially focusing on B lymphocyte function in future.

Osteopontin transgenic mice have already been made by Chiba et al. [16] and Isoda et al. [17] using an immunoglobulin enhancer (Emu)/SV40 promoter and a cytomegalovirus enhancer/chicken β-actin promoter, respectively. In the former model, osteopontin was expressed abundantly in the lymphoid tissues such as the thymus and spleen [16], while the expression was found ubiquitously in all tissues in the latter model [17]. Both models were used to study the role of osteopontin in the development of atherosclerosis [16,17], but histological features of the liver were not examined in these models. Our transgenic mice are the first model showing osteopontin expression exclusively in the liver, and may be available for studying the significance of Th1 immune response in the development of chronic liver diseases.

Toyonaga et al. [11] reported the transgenic mice expressing IFN-γ in the liver using a pLG1-SAP vector. Histological findings of the liver in these mice were comparable to those of our transgenic mice, in which focal necrotic lesions with lymphocyte infiltration developed spontaneously in the liver. IFN-γ is produced by T lymphocytes following stimulation by IL-18 and IL-12 [18], while osteopontin is considered to exist in the up-stream of IL-18 and IL-12 in the cytokine network [10]. IFN-γ production is shown to be up-regulated in T lymphocytes in the spleen of the IL-18 transgenic mice [19]. Also, it is reported that lymphocyte infiltration developed in the central nervous system and pancreas with increased expression of IFN-γ in the transgenic mice expressing IL-12 in astrocytes [20,21] and pancreatic β cells [22], respectively. In our previous experiments, IFN-γ and IL-10, representative cytokines involved in Th1 and Th2 immune responses, respectively, were undetectable in the plasma of both transgenic and control mice [23]. When the mice were given intravenous injections of concanavalin-A, a mitogenic plant lectin for T lymphocytes, massive liver necrosis developed following up-regulation of plasma concentrations of IFN-γ and IL-10. However, the extent of liver necrosis and the up-regulation of plasma IFN-γ concentration were greater in the transgenic mice than in the control mice, while the up-regulation of plasma IL-10 concentration was smaller in the transgenic mice than in the control mice [23], suggesting that Th1 cytokine network of IL18, IL-12, and IFN-γ may be activated in the liver of the osteopontin transgenic mice as was in the transgenic mice expressing IL-18 and IL-12 [19–22]. These matters should be investigated more precisely in future.

In conclusion, transgenic mice expressing osteopontin exclusively in hepatocytes may be useful as a model of autoimmune hepatitis.

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