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Original Article

The relationship between spontaneous cystic degeneration and pseudocapillarization in sinusoids in the liver of aged Sprague-Dawley rats

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Abstract: Cystic degeneration (CD) in the liver is a cyst-like lesion composed of one or more pseudocysts lacking lining cells, occurring spontaneously in rats older than 12 months, with a male predilection. In this study, 32 CDs were identified in 23 out of 104 non-treated, control male Sprague-Dawley rats from two combined chronic toxicity and carcinogenicity studies with agrochemicals. They were examined histologically, histochemically, and immunohistochemically to assess the pathogenesis and pathological significance of CD, focusing on pseudocapillarization in aged rat liver. Pseudocapillarization refers to age-related capillarization of hepatic sinusoids and is distinct from sinusoidal capillarization observed in hepatic cirrhosis. Both CD and pseudocapillarization, characterized by factor VIII-related antigen expression, were primarily noted in the periportal regions of the rat liver. CD areas exhibited enhanced vimentin expression in a diffuse linear pattern in their septa with occasional focal linear α-smooth muscle actin expression and the fluid containing hyaluronic acid accumulated in their lumen that are thought to be formed by hepatocellular apoptosis. These findings suggest a series of reactive changes associated with hepatocellular apoptosis due to pseudocapillarization in the sinusoids. In conclusion, spontaneous CD in rat liver is not a degenerative lesion or cystic enlargement of stellate cells, but a structural abnormality in pre-existing liver tissue resulting from aging-related changes in sinusoidal endothelial cells and hepatocytes. Pseudocapillarization of sinusoids is considered a precursor lesion of CD in the rat liver. (DOI: 10.1293/tox.2024-0034; J Toxicol Pathol 2025; 38: 27–36)

Key words: rat, cystic degeneration, spongiosis hepatis, liver, pseudocapillarization, aging

Introduction

Cystic degeneration (CD) in the rat liver is a cyst-like lesion that occurs spontaneously in rats older than 12 months, with a male predilection[1, 2]. This lesion was first reported in *N*-nitrosomorpholine-treated rat livers as spongiosis hepatis[3]. Despite being known for 40 years, the pathogenesis and pathological significance of CD remains unclear. Additionally, the increasing incidence of CD in rat carcinogenicity studies poses potential challenges for human risk assessment.

Histologically, CD areas consist of one or more pseudocysts lined by basement membrane (BM) and/or collagen fibers, but not by cells[3, 4]. Most pseudocysts contain acid mucopolysaccharide (AM)[3]. Additionally, most CDs are multilocular lesions, although some are theoretically composed of a single pseudocyst. The constituent cells in CD areas include hepatic stellate cells and fibroblast-like cells[3], but their origin remains unknown. CD is often considered a degenerative lesion[1] or cystic enlargement[2] of stellate cells in the liver. Despite their stellate cell origin, CD areas occasionally become larger than the hepatic lobules. Additionally, CD areas contain fluid and/or AM, stellate cells cannot retain fluid because of their role in supporting sinusoids as liver specific pericytes[5]. Therefore, it is unlikely that CD originates within or is predominantly composed of stellate cells.

Pseudocapillarization is characterized by the thickening of sinusoidal endothelial cells (SECs), a decreased number and diameter of fenestrations in SECs, development of BM, and increased collagen deposition in the space of Disse[6]. Pseudocapillarization refers to the capillarization of liver sinusoids, but is termed "pseudo" to distinguish it from sinusoidal capillarization in liver cirrhosis[6]. CD is an age-related lesion of the rat liver[1, 2] as described above. Similarly, pseudocapillarization in sinusoids is also an age-related change in the structure and morphology of the liver in rats[6]. Both CD and pseudocapillarization in sinusoids common age-related changes in BM formation and collagen

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deposition.

This study aimed to determine the pathogenesis and pathological significance of CD by performing histological, histochemical, and immunohistochemical examinations of CD and non-CD areas in the livers of aged rats with spontaneous CDs, with a focus on pseudocapillarization in sinusoids.

Materials and Methods

Thirty-two CDs found in 23 of 104 non-treated, control male Sprague-Dawley rats (Jackson Laboratories Japan, Atsugi, Japan) were used in this study. All rats examined were scheduled for sacrifice at 109 weeks of age in two combined chronic toxicity and carcinogenicity studies with agrochemicals at the Institute of Environmental Toxicology (IET), which is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International. The procedures for handling and caring for the animals used in this study were approved by the Guidelines for Animal Experimentation issued by the Japanese Association for Laboratory Animal Science (JALAS)[7] and the Code of Ethics for Animal Experimentation of IET (IET Approval No. AC14137, AC14138).

Archived 10% neutral-buffered formalin-fixed, paraffin-embedded liver tissues from the medial and left lobes were used for histopathological examination in this study. Paraffin sections of all CDs from 23 animals were stained with hematoxylin and eosin (HE). These HE-stained sections were examined histopathologically. CD was defined as a multilocular cystic lesion[2, 4]. The CD areas were classified by size into small (less than a hepatic lobule), medium (approximately the size of a hepatic lobule), and large (more than a hepatic lobule). The number of small CDs at each site within the hepatic lobule was counted. Moreover, special staining and immunostaining were applied to additional paraffin sections from nine small CD areas exhibiting typical CD histology in nine rats. The details of the histochemical staining methods and antibodies used for immunostaining are presented in Tables 1 and 2, respectively. For alcian blue (AB) staining, hyaluronidase digestion (from bovine testes, Nacalai Tesque, Inc., Kyoto, Japan) was performed to identify the contents within the CD areas. In immunostainings, EnVision + System-HRP anti-rabbit or mouse IgG (Agilent Technologies, Santa Clara, CA, USA) was used as the secondary antibody and the immunoproducts were visualized with 3,3′-diaminobenzidine as chromogen. Nuclear staining was performed using Mayer's hematoxylin. All stained sections were examined histologically using light microscopy. Factor VIII-related antigen (FVIII-RAg) immunostaining was employed as a histological marker for pseudocapillarization in the sinusoids.

Table 1. Summary of Histochemical Staining Methods

Histochemical staining methods	Staining target	Purpose of staining
Schmorl reaction	Lipofuscin	Detection of indicator of aging and/or oxidative stress
One step trichrome staining Watanabe's silver impregnation staining	Collagen fibers Reticular fibers	Identification of non-cellular components in the septa of CD areas
AB staining (pH 2.5) with testicular hyaluronidase digestion	Acid mucopolysaccharide (hyaluronic acid)	Identification of contents within CD areas

AB: alcian blue; CD: cystic degeneration.

Table 2. The Antibodies Used

Antibodies	Source (clone)	Dilution ratio	Pretreatment	General reactivity in the rat hepatic sinusoids
Anti-human vimentin (Rm)[a)]	Abcam plc, Cambridge, UK (EPR3776)	1:300	Microwave oven heating (95°C, 5 min., citrate buffer, pH 6.0)	SECs, Kupffer cells, Stellate cells
Anti-human α-SMA (Mm)	Agilent Technologies Ltd., Santa Clara, USA (1A4)	1:50	Microwave oven heating (95°C, 5 min., citrate buffer, pH 6.0)	Activated stellate cells
Anti-rat monocytes/ macrophages (Mm)	Bio-Rad AbD Serotec Ltd., Kidlington, UK (ED-1)	1:50	Protenase K (RT, 15 min.)	Kupffer cells, Monocytes
Anti-human FVIII-RAg (Mm)	BioGenex, Fremont, USA (F8 2.2.9)	prediluted	Protenase K (RT, 15 min.)	None
Anti-rat PCNA (Mm)	Agilent Technologies Ltd., Santa Clara, USA (PC10)	1:200	Microwave oven heating (95°C, 5 min.)	Proliferating cells (Max. in DNA replication phase; S phase)
Anti-rat sinusoidal endothelium (Mm)	IBL Co., Fujioka, Japan (SE-1)	1:5	Protenase K (RT, 15 min.)	SECs

[a)](Rm): Rabbit monoclonal, (Mm): Mouse monoclonal. α-SMA: α-smooth muscle actin; FVIII-RAg: factor VIII-related antigen; PCNA: proliferating cell nuclear antigen; SECs: sinusoidal endothelial cells; RT: room temperature.

Results

The number of small CD per occurrence site within hepatic lobule

The number of CD occurrences ranged from one to three per rat. Among the 32 CDs, 15 were classified as small. Small CDs occurred in the periportal region in 11 of 15 (73%) and in the subcapsular region in 4 of 15 (27%). The number of moderate and large CDs were 7 and 10, respectively. For these moderate and large size CDs, their intralobular distributions could not be determined due to the absence of central veins or portal triads around the CD areas. No CDs were detected in the centrilobular region.

Histopathology

CD area:

All CDs examined were multilocular lesions consisting of three or more pseudocysts. The pseudocyst wall corresponded to the septa of the CD areas. The pseudocysts were empty or contained eosinophilic homogeneous, flocculent, or granular material (Fig. 1a and 1b) and were sometimes, accompanied by minimal to slight hemorrhage and/or leukocytes. The cellularity within the CD area was usually low (Fig. 1b). However, atrophic hepatocytes (Fig. 1a) and/or condensed erythrocytes (Fig. 1b) were occasionally observed within CD areas and their septa, respectively. Most constituent cells in the septa had spindle shaped, hyperchromatic nuclei, and inconspicuous cytoplasm (Fig. 1b); however, some cells with oval and hypochromatic nuclei were also occasionally observed (Fig. 1b). Although rare, hepatocellular apoptosis has been detected within or around large CDs. Similarly, in small CD areas, apoptotic hepatocytes were observed within pseudocysts (Fig. 1c), surrounded by hypereosinophilic hepatocytes with condensed nuclei (Fig. 1c). Slit formation was also observed in the septa of the CD area (Fig. 1c).

Non-CD area:

In only one case, small cystic spaces were observed in the periportal region (Fig. 1d) and contained apoptotic hepatocytes, eosinophilic materials, or inflammatory cells (Fig. 1d). However, no noteworthy lesions were observed in the periportal regions of the liver in the other animals examined.

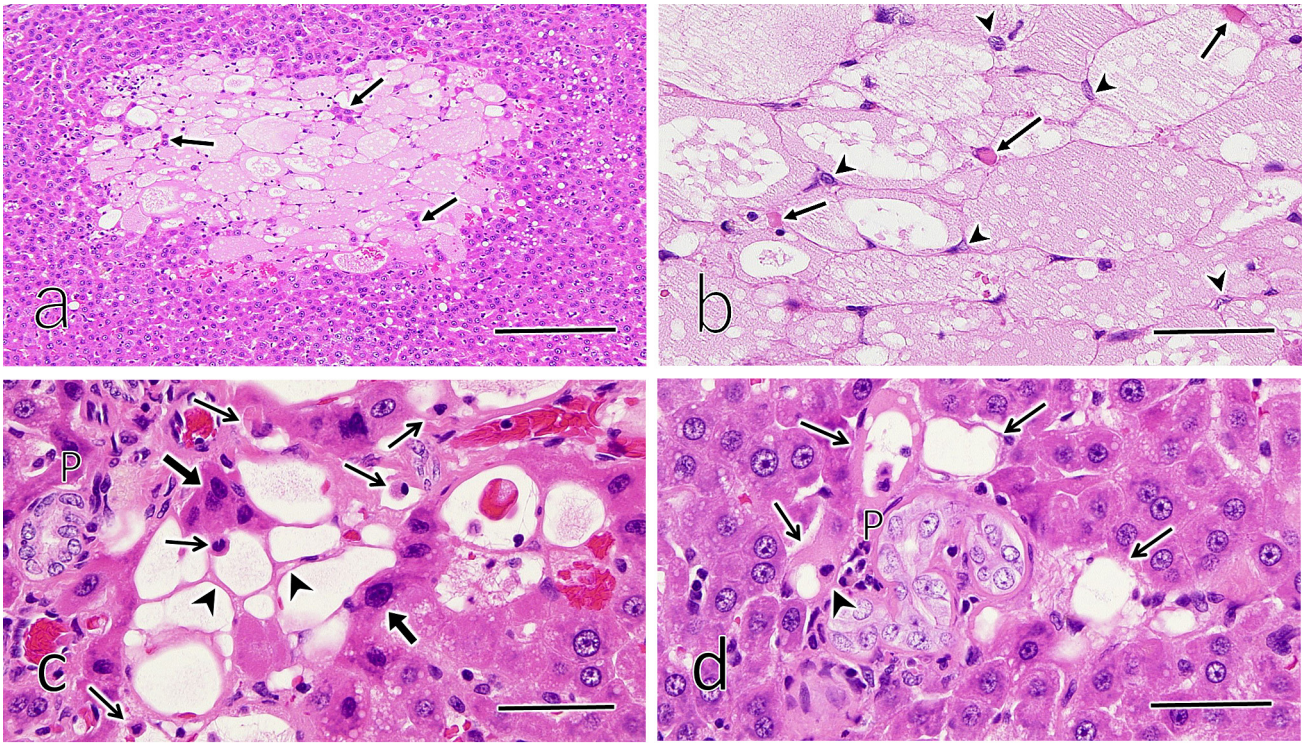

Fig. 1. (a) A typical histology of large cystic degeneration (CD) area. CD area was composed of many pseudocysts and some hepatocytes (arrows) were present within CD area. (b) High magnification of Fig. 1 (a). The pseudocysts without lining cells show various sizes and morphologies and contained fine granular to flocculent eosinophilic material. The septa of CD area were very thin and condensed erythrocytes (arrows) were noted. Two types of constituent cells could be recognized as hyperchromatic cells and hypochromatic cells (arrowheads) based on the amount of chromatin. (c) Apoptotic cells were observed within small CD area (thin arrows). CD area was surrounded by hypereosinophilic hepatocytes with condensed nuclei (thick arrows) and slit formation (arrowheads) was observed in the septa. (d) Small cystic spaces (arrows) were detected around the portal tract in non-CD area. Some of those spaces contained apoptotic hepatocyte (arrowhead), a few inflammatory cells and eosinophilic materials. P: portal tract, Bar=(a) 200 µm, (b–d) 50 µm. HE stain.

Histochemistry

CD area:

Collagen and reticular fibers in the septa were visualized using trichrome staining and Watanabe's silver impregnation staining, respectively. Their quantities varied from case to case. The contents within the pseudocysts were AB-positive, with staining intensity decreasing with increasing CD area. Additionally, AB-positive materials disappeared after digestion with hyaluronidase derived from the bovine testes. Schmorl's reaction-positive substances were not detected in any of the cells.

Non-CD area:

The AB-positive material were rarely detected around the portal area (Fig. 2a). Lipofuscin deposition by the Schmorl's reaction was more abundant in sinusoidal lining cells than in hepatocytes across all cases examined (Fig. 2b). Pigmented sinusoidal lining cells, most prominently SECs, were identified based on their location, cytological characteristics, and ED-1-negative immunostaining results. Lipofuscin deposition in SECs was notably observed in the periportal regions (Fig. 2b).

Immunohistochemistry

The immunostaining results for the septa in CD areas and sinusoidal walls in non-CD areas are summarized in Table 3.

CD area:

Vimentin (Fig. 3a) and α-smooth muscle actin (α-SMA) (Fig. 3b)-positive cells were detected linearly in the septa of CD areas. Vimentin expression showed a diffuse pattern (Fig. 3a) and was enhanced compared to surrounding liver tissue, while α-SMA expression pattern was focal or multifocal (Fig. 3b), but rarely annular. In contrast, FVIII-RAg positivity was observed in an annular pattern (Fig. 3c). ED-1 positive cells appeared spindle-shaped in the septa and round-shaped in the lumen of CD areas (Fig. 3d), with

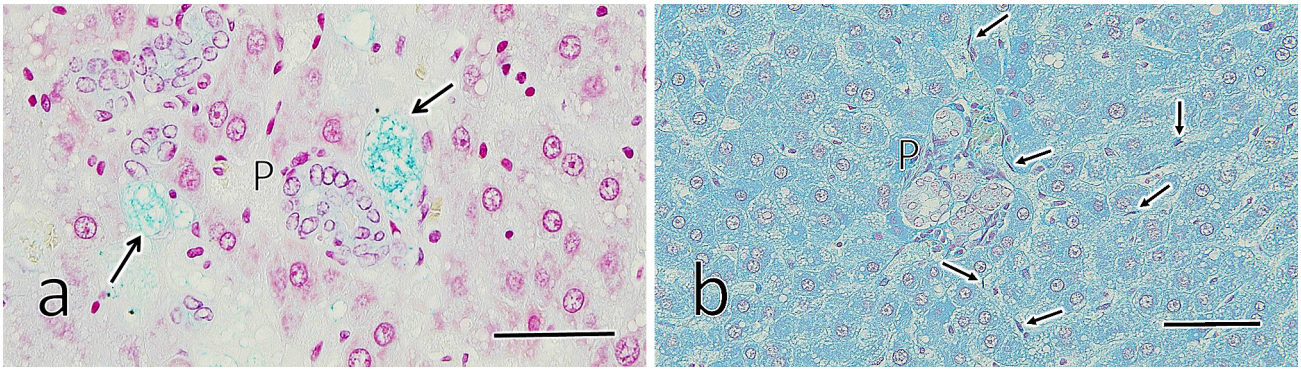

Fig. 2. (a) Alcian blue positive materials (arrows) accumulated in the cystic spaces around the portal tract (P), (b) Lipofuscin deposited in sinusoidal endothelial cells (arrows) but was unclear in Kupffer cells and hepatocytes. Bar=(a, b) 50 μm. (a) Alcian blue stain, (b) Schmorl reaction.

Table 3. Summary of Results of Immunostainings

Markers	Septa in CD areas	Sinusoidal walls in non-CD areas
Vimentin	+++ (diffuse linear)	+ (diffuse cytoplasmic)
α-SMA	+ (focal linear, annular[b])	-
ED-1[a]	+ (diffuse cytoplasmic)	+ (diffuse cytoplasmic)
FVIII-RAg	+ (annular)	+[c] (annular)
PCNA	-	-
SE-1[a]	-	+++ (diffuse linear)

Staining intensity: -, none; ± , minimal; +, slight; ++, moderate; +++, marked.
( ): Staining pattern. a) clone name, b) rare pattern, c) occasionally in the periportal region. CD: cystic degeneration; α-SMA: α-smooth muscle actin; FVIII-RAg: factor VIII-related antigen; PCNA: proliferating cell nuclear antigen.

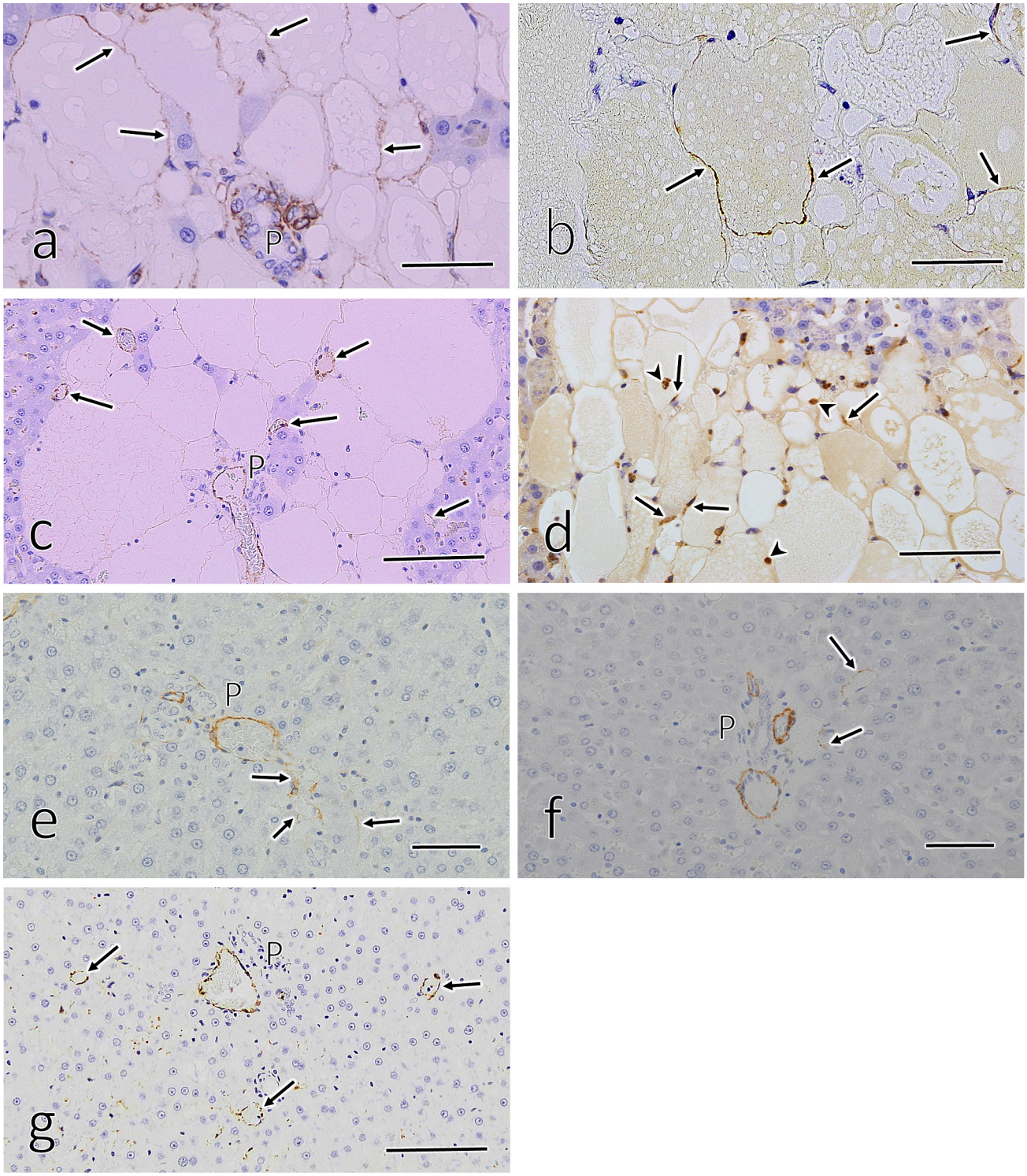

Fig. 3. Immunostaining results of CD area. (a) Vimentin expression (arrows) was detected diffuse linearly in the septa of CD area. (b) α-SMA immunoreactivity (arrows) were observed multifocal linearly within CD area. (c) Capillarized sinusoids (arrows) were detected in CD area. (d) Elongated macrophages (arrows) in the septa of CD area and round-shaped macrophages (arrowheads) in the lumen of CD area. In non-CD areas, α-SMA expression (arrows) was detected in sinusoids around the portal tract and their expression patterns were associated with cellular reaction (e) or not (f). (g) FVIII-RAg expression (arrows) was observed in sinusoids in the perilobular region in non-CD area. Immunostainings with anti-vimentin (a), α-SMA (b, e, f), FVIII-RAg (c, g), and macrophage (clone name: ED-1) (d) antibodies. P: portal tract. Bar=(a, b, e, f) 50 μm, (c, d, g) 100 μm.

numbers comparable to those in the non-CD areas. No SE-1 (anti-rat sinusoidal endothelium antibody) or proliferating cell nuclear antigen (PCNA)-positive cells were found within the CD areas.

Non-CD area:

α-SMA expression was detected in one to five periportal regions in all cases (Fig. 3e and 3f). These α-SMA expression patterns were either associated with cellular reaction (Fig. 3e) or not (Fig. 3f) and were not detected outside the periportal regions. FVIII-RAg expression was observed in an annular pattern (Fig. 3g), in the CD areas, and its expression was observed in three or more periportal regions in all cases. In cases with extensive or strong FVIII-RAg expression, it was also observed in the centrilobular regions and/or within the hepatic lobules, although to a lesser extent than in the periportal regions. Diffuse SE-1 positivity was observed along the sinusoids, and PCNA-positive hepatocytes were seldom observed. Moreover, no linear expression of vimentin was observed in the sinusoids.

Discussion

CD is a degenerative lesion[1] or cystic enlargement[2] of stellate cells in the rat liver. However, this conclusion appears inappropriate due to the lesion size and histological characteristics such as fluid retention containing AM. CD areas show low cellularity and the same noncellular components[4] as normal liver tissue[8]. Additionally, PCNA immunostaining revealed low proliferative activity in cells within the CD areas. Based on these findings, we hypothesized that CD is composed of pre-existing liver tissue rather than stellate cell-based changes or newly formed tissue. To test this hypothesis, we focused on the relationship between CD and pseudocapillarization in sinusoids in aged male rat livers. Both CD and pseudocapillarization in sinusoids are age-related changes involving BM formation and/or collagen deposition in the rat liver, and CD occurs spontaneously with relatively high incidence in male rats. Additionally, non-CD areas were examined to detect CD precursor lesions.

An open fenestration without a diaphragm or BM is typical feature of SECs[9]. However, with age, SECs begin to show pseudocapillarization, characterized by thickening of SECs, accompanied by reduced fenestrations, BM development, and increased collagen deposition in the space of Disse[6]. At the light microscopy level, FVIII-RAg expression secondary to BM formation, serves as a marker for capillarization in rat[6] and human[10] liver sinusoids. Additionally, transmission electron microscopy (TEM) is more sensitive than immunohistochemistry for laminin or Masson's trichrome staining of collagen fibers in detecting BM[11]. Therefore, pseudocapillarization in sinusoids may occur more extensively in aged rat livers than in the FVIII-RAg immunostaining results.

This study showed that both CD and FVIII-RAg expression in the sinusoids, a histological marker for pseudocapillarization, occurs predominantly in the periportal region of the liver. Although no lobular gradient in FVIII-RAg expression was noted in aged rat liver[6], its initial expression was restricted to the periportal regions in this study.

Thus, we focused on SECs that formed sinusoids, particularly in the periportal region, to clarify the relationship between CD and pseudocapillarization in the sinusoids. The constituent cells in the septa of CD areas are believed to be stellate cells and fibroblast-like cells[3]. However, the origin of these fibroblast-like cells remains unclear. Ultrastructurally, fibroblast-like cells within CD areas are characterized by extremely elongated cytoplasmic processes[3, 4], which contact neighboring cells to form large cavities[4]. Three-dimensional analysis showed that the membranous processes of SECs extend to neighboring sinusoids at branching points[12]. This ultrastructural feature of SECs is consistent with the fibroblast-like cells observed in the septa of CD areas.

SEC was considered a candidate for fibroblast-like cells in the septa of CD areas due to the presence of capillarized sinusoids within CD areas, as revealed by this study and the TEM findings described above. Additionally, the presence of condensed erythrocytes and elongated Kupffer cells in the septa of CD areas provides indirect evidence of SECs in these areas. However, the septa of the CD areas in this study were negative for SE-1, a specific marker of rat SECs[13]. The septa of CD areas are lined with BM and/or collagen fibers[3] and SE-1 is a cell surface protein[13]. SECs lose their unique native characteristics upon cultivation for more than 1-2 days, showing significant differences from native SECs. Moreover, SECs exhibit phenotypic and functional variations in healthy or diseased liver[14]. Therefore, the absence of SE-1 in CD areas suggest that the surface of SECs is not exposed to outside or shows phenotypic changes due to environmental alterations. The slit formation observed in the septa of the small CD areas suggests that some septa were formed by adhesion between the septa. In capillarized sinusoids, the BM and collagen fibers are formed beneath the SECs, that is, the space of Disse. Experimental results suggest SEC involvement in BM formation during sinusoidal capillarization in liver disease[15]. Thus, BM formation within CD areas indicates SEC involvement, and the presence of pseudocysts lined with the BM suggests that these pseudocysts may originate from the space of Disse in capillarized sinusoids.

In the present study, vimentin expression was enhanced and exhibited a diffuse linear pattern in the septa of the CD area compared with the sinusoids in the surrounding liver tissue. Since vascular endothelial cells increase vimentin expression under hypoxic conditions[16], this enhancement in vimentin expression could also be related to hypoxic conditions in SECs. Vimentin expression can also be enhanced by changes in cell shape or mortility[17] and by providing protection against mechanical stress[18]. In addition, the expression pattern of vimentin in the septa of CD areas further indicated that the septa of CD areas are formed by adjacent cells and that their constituent cells have the ability to retain fluid. SECs can also retain fluid because SEC, but not stellate cells, form the wall of the sinusoids. Therefore, fibroblast-

like cells and/or vimentin-positive cells in the septa of CD areas are likely to be SECs, considering that SECs occupy a large portion of the cell membrane surface of the sinusoidal lining cells[9], although there is a possibility of involvement of stellate cells and Kupffer cells.

On the other hand, α-SMA, which are not expressed in normal rat liver sinusoidal lining cells including stellate cells[19], was observed only in the septa of CD areas. Typically, α-SMA expression in hepatic stellate cells indicate their activation, which leads to collagen production[20]. In aged rat livers, pseudocapillarization of sinusoids leads to α-SMA expression in stellate cells, resulting in perisinusoidal fibrosis[5]. Experimental results have demonstrated that non-capillarized SECs prevent stellate cell activation, whereas capillarized SECs lose this effect[21]. Therefore, α-SMA expression in CD areas likely reflects stellate cell activation through pseudocapillarization in sinusoids, although no clear correlation between α-SMA expression and collagen production within CD areas at light microscopy level.

The contents within the CD areas were considered hyaluronic acid (HA), which is a type of AM, rather than other AMs, based on AB staining after digestion by testicular hyaluronidase and its localization in non-cartilaginous tissue. HA, synthesized by hepatic stellate cells as along with collagens[22] is prominently produced during early injury in the liver[23]. This HA production by stellate cells may be a reactive response to hepatocellular injury, as reported by Karbe and Kerlin[24]. Moreover, over 90% of serum HA is degraded by SECs[25] and the HA removal rate in the liver reflects SEC function[26]. High serum HA levels in humans correlate with morphological changes in SECs, such as BM formation and FVIII-RAg expression[10]. Pseudocapillarization in sinusoids indicates a lowered function of SECs in the liver.

Although rare, hepatocellular apoptosis was observed within or adjacent to the CD areas. It is possible that hepatocellular apoptosis occurs in the livers of aged rats with CDs as a result of hepatocellular injury, unlike physiological apoptosis in the centrilobular region[27]. Moreover, the relationship between CD and degeneration or necrosis of hepatocytes has been previously reported, including ultrastructural degenerative changes in hepatocytes left behind within CD areas[3, 4] and CD occurrence along with or within necrotic areas in the rat liver[2, 28]. Karbe and Kerlin assumed that hepatocellular dropout occurs following focal damage in CD areas based on the finding that CD does not compress the surrounding liver tissues and that the surrounding architecture is not distorted[24].

Pseudocapillarization in the sinusoids induces hepatocellular apoptosis by restricting the availability of oxygen and other nutrients in hepatocytes[6]. Additionally, pseudocapillarization acts as a barrier to hepatocellular regeneration[29] and the regenerative capacity of hepatocytes declines with age[30]. Consequently, the spaces remaining after hepatocellular apoptosis were not replaced by regenerated hepatocytes. In fact, PCNA-positive cells are rare among hepatocytes in non-CD areas of the livers of rats with CD. Pseudocapillarization in sinusoids may contribute to hepatocellular apoptosis in the liver of rats with CD as both pseudocapillarization and CD have common occurrence sites, as revealed by the results of this study. This hepatocellular apoptosis may also trigger HA secretion via α-SMA expression in stellate cells. HA accumulates the remaining spaces after hepatocellular apoptosis, and fluid gradually accumulates in the spaces owing to the high water molecule-binding capacity of HA[31]. This finding suggests that pseudocysts represent histological dilatation of the space of Disse in capillarized sinusoids.

CD in the liver is induced by both genotoxic and non-genotoxic carcinogens[28], but the cause is not directly related to the genotoxic or carcinogenic potential of the compound[24]. Oxidative stress (OS), defined as an imbalance between the production of reactive oxygen species (ROS) and antioxidant defense mechanisms, represents the underlying cause of the increased risk of hepatocarcinogenesis, irrespective of genotoxic or non-genotoxic carcinogen[32]. Additionally, OS plays an important role in aging[33] as well as hepatocarcinogenesis. Interestingly, CD is observed adjacent to or within hepatocellular proliferative lesions such as altered hepatocellular foci (foci)[3, 34], nodular hepatocellular hyperplasia[35], hepatocellular adenoma[3, 4], and hepatocellular carcinoma[3, 4] as well as in non-lesioned areas in the liver. Experimentally induced nodular hepatocellular hyperplasia[36], hepatocellular adenoma[37], and hepatocellular carcinoma[37] have also been reported to show capillarization in the sinusoids. CD is more likely to occur within these hepatocellular proliferative lesions, except for foci, because of their occurrence of capillarization in their sinusoids. However, the occurrence of CD associated with induced hepatocellular proliferative lesions is not limited to lesional areas, but is also found around the lesions. Accordingly, CD associated with induced hepatocellular proliferative lesions may be the result of factors such as OS affecting not only hepatocytes but also sinusoidal lining cells during the development of their proliferative lesions, in addition to the capillarization of these lesions themselves. Studies examining the effects of oxidants on the liver have demonstrated that 3-nitrotyrosin, which is an OS marker, is increased in the sinusoids, particularly in the periportal region[38]. This periportal-specific occurrence was also noted in both CD and pseudocapillarization of the sinusoids.

Similarly, lipofuscin can be used as a marker for OS and aging[39]. In this study, lipofuscin deposition was consistently more pronounced in SECs than in hepatocytes, although lipofuscin deposition in hepatocytes is considered a characteristic of age-related changes in the liver as well as pseudocapillarization[40]. Age-related changes in SECs precede those in hepatocytes[41] and are more pronounced than those in hepatocytes[41]. These findings are consistent with the fact that OS selectively injures SECs[38] and are evidenced by the fact that lipofuscin deposition was more pronounced in SECs in the periportal region in this study. As the antioxidant capacity in rats is not affected by age, OS-induced damage in rats is due to an age-related increase in systemic ROS[42]. Human females are known to be less susceptible

to OS than males under physiological conditions, and one of the factors that plays a role in this antioxidant effect is estrogen[43]. In fact, 17β-estradiol, which is a potent endogenous antioxidant, attenuated experimentally all age-related changes including histological changes in the rat liver by its protective effect for OS[44]. This may explain why CDs occur preferentially in males.

Taken together, the aging of SECs in the periportal regions appears to play a significant role in the pathogenesis of CD, with SEC being the primary cells in CD occurrence rather than stellate cells. Additionally, OS production may contribute to the development of CD.

Based on the results of this study, we hypothesize the pathogenesis of spontaneous CD is as illustrated in Fig. 4. The process is proposed as follows: First, aging leads to pseudocapillarization in liver sinusoids, followed by decreased supply of oxygen or nutrients secondary to pseudocapillarization in sinusoids, causing hepatocellular dropout by apoptosis. However, the regenerative capacity of hepatocytes is diminished due to aging, preventing hepatocellular regeneration. Despite the loss of hepatocytes, the fundamental structure of the liver was preserved, because apoptosis does not provoke significant inflammation or tissue destruction. Subsequently, HA produced by hepatic stellate cells, activated by pseudocapillarization in sinusoids and/or hepatocellular apoptosis, accumulates in the space left after hepatocellular apoptosis (i.e., the space of Disse) and leads to fluid retention due to HA accumulation, resulting in CD formation. Thus, CD is triggered by pseudocapillarization in sinusoids and is considered to be caused by pre-existing liver tissue, but not stellate cell-based changes or newly formed tissue.

In conclusion, spontaneous CD in the rat liver is not a degenerative lesion or cystic enlargement of hepatic stellate cells, but rather a structural abnormality in pre-existing liver tissue caused by aging-related changes in SECs and hepatocytes. Pseudocapillarization of sinusoids is considered a precursor lesion of CD in the rat liver.

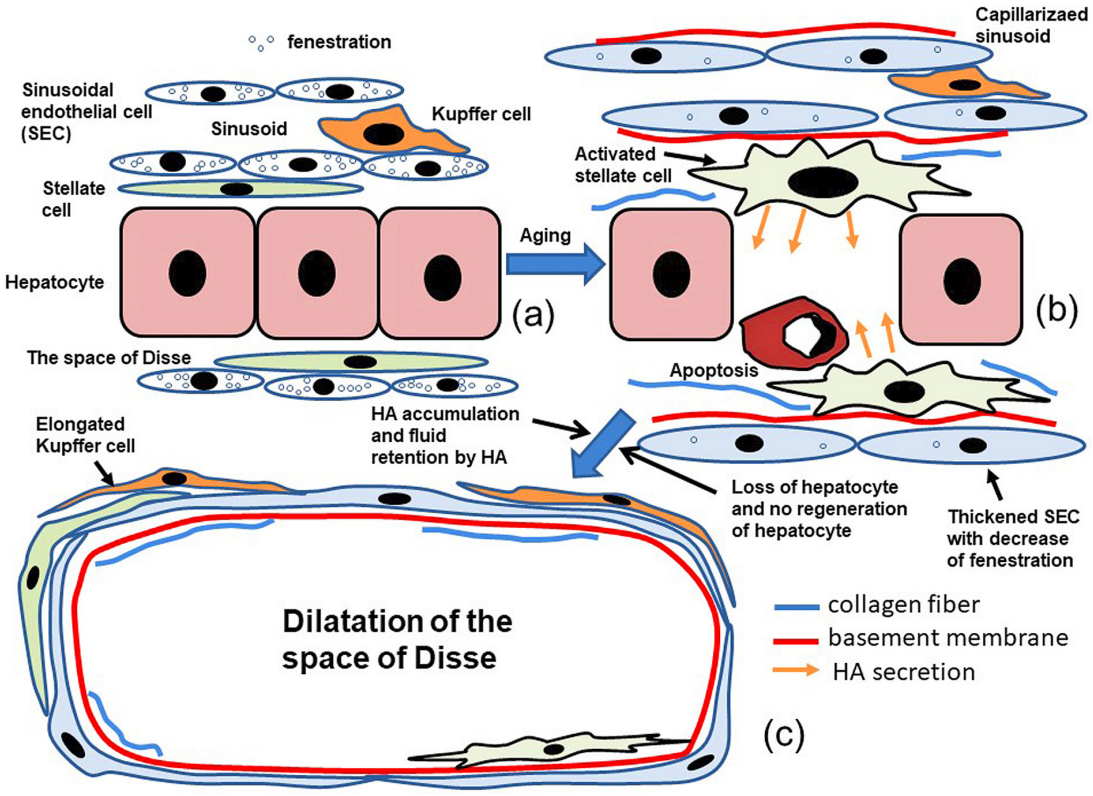

Fig. 4. A shematic drawing showing the possible pathogenesis of CD. (a) Normal histological relationship between sinusoids, hepatic cord, and the space of Disse. Sinusoidal endothelial cells (SECs) have many fenestrations and quiescent stellate cells reside in the space of Disse. (b) Aging changes in the sinusoids and the space of Disse. Increased deposition of collagen fibers (blue lines) and secretion of HA (orange arrows) by activated stellate cells and BM formation (red lines) by thickened SECs without fenestrations in the space of Disse and hepatocellular apoptosis. (c) The space left by hepatocellular dropout owing to hepatocellular apoptosis was dilated by fluid retention after HA accumulation. The pseudocyst wall consists of SECs (blue cells) lined by BM and/or collagen fibers. Kupffer cells (orange cells) and stellate cells (green cells) are enclosed and stretched in the septa due to compression secondary to HA accumulation and fluid retention. SECs, sinusoidal endothelial cells; HA, hyaluronic acid.

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