The Role of Heparan Sulfate Proteoglycan, TGF-β1, and Kupffer Cells in Oval Cell Differentiation after Galactosamine (GaIN)-Administration in Mini Rats

Koji Uetsuka, Michio Suzuki, Hiroyuki Nakayama, and Kunio Doi
Department of Veterinary Pathology, Faculty of Agriculture, The University of Tokyo

Abstract: Six-week-old male Mini rats were intraperitoneally given 1,000 mg/kg of D-galactosamine hydrochloride (GaIN) once a week for 4 consecutive weeks and killed at 1, 2, 3, and 5 days (D) and 1, 2, 3, and 4 weeks (W) after the first GaIN-administration, respectively. Proliferation of oval cells was observed at and after 3 D. Around them, prominent deposition of heparan sulfate proteoglycans was observed throughout the experimental period. Positive stainability for transforming growth factor (TGF)-β1 was detected in the cytoplasm of both bile duct epithelial cells in the Glisson's capsules and proliferating oval cells throughout the experimental period, and this may suggest an internalization of latent-type TGF-β1 in oval cells as well as bile duct epithelial cells. These results, in turn, indicate the differentiation of oval cells into bile duct epithelial cells. On the other hand, the number of Kupffer cells positive for ED2 peaked at 2D and thereafter decreased gradually, and no spatial relationship was detected between Kupffer cells and proliferating oval cells. (J Toxicol Pathol 1997; 10: 205–209)

Key words: Mini rat, Galactosamine, Oval cell, Heparan sulfate proteoglycan, TGF-β1, Kupffer cell

Introduction

The rat of Jcl: Wistar-TgN (ARGHGEN) 1Nts strain (Mini rat) is a Wistar rat-derived transgenic one in which the expression of growth hormone gene is suppressed by the insertion of an antisense transgene1,2. In Mini rats, prolonged proliferation of oval cells3 was observed after a single administration of D-galactosamine hydrochloride (GaIN)4, and therefore this strain of rats is considered to be a useful tool for the investigation of oval cell differentiation.

In our previous studies on acute4 and subacute5 GaIN-induced hepatitis in Mini rats, it was suggested that Ito cells might be involved in the proliferation and/or differentiation of oval cells into bile duct epithelial cells through the production of such extracellular matrix materials as laminin and type IV collagen, important components of basement membrane6. In this study, we investigated the changes in the deposition of heparan sulfate proteoglycan7, one of the major components of basement membrane, in the expression of transforming growth factor (TGF)-β1, and in the number of Kupffer cells located in the sinusoidal linings after GaIN-administration in relation to oval cell differentiation.

Materials and Methods

Fifty-six 6-week-old male rats of the Jcl: Wistar-TgN (ARGHGEN) 1Nts strain (Mini rats)1,2 (body weight 95–115 g), which were kindly given from NT Science Co. (Tokyo, Japan), were used. The animals were confirmed to be free from specific pathogens. They were kept under controlled conditions (temperature, 23±2°C; relative humidity, 55±5%) in an isolator caging system (Niki Shoji Co., Tokyo) and were fed pellets (MF, Oriental Yeast Co., Tokyo) and water ad libitum.

Thirty-two rats were intraperitoneally (ip) administered with 1,000 mg/kg b.w. of D-galactosamine hydrochloride (GaIN) (Sigma Chemical Co., MO, USA) once a week (on Monday) for 4 consecutive weeks. The injection volume was adjusted to 10 ml/kg b.w. using 0.9% saline. The remaining 24 rats received the same volume of 0.9% saline and served as controls. Four rats of the GaIN-group and 3 controls were killed by exanguination under ether anesthesia at 1, 2, 3, and 5 days and 1, 2, 3, and 4 weeks after the first GaIN-administration, respectively. Slices of the liver were frozen in dry ice-hexane for immunohistochemistry. The remaining liver was fixed in 10% neutral-buffered formalin, and paraffin sections (4 μm) were stained with hematoxylin and eosin (HE).

Cryosections were fixed in acetone at -20°C for 10 min before immunohistochemical staining. Antibodies against heparan sulfate proteoglycan (Seikagaku Co., Tokyo, Japan), were used. The animals were confirmed to be free from specific pathogens. They were kept under controlled conditions (temperature, 23±2°C; relative humidity, 55±5%) in an isolator caging system (Niki Shoji Co., Tokyo) and were fed pellets (MF, Oriental Yeast Co., Tokyo) and water ad libitum.

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Proliferation of small round oval cells was first observed around the Glisson's capsules at 3 days after the first GaIN-administration (3D). At 7D, oval cells proliferated in rows,
in clusters or in ductular structures in the whole hepatic lobules. A large number of them proliferated making up ductular structures at 2-4 weeks after the first GalN-administration (2-4W).

Immunohistochemically, heparan sulphate proteoglycans were clearly observed around the proliferating oval cells near the Glisson's capsule at 3D (Fig. 1a), and they were also observed around the diffusely proliferating oval cells in the hepatic lobules at 7D (Fig. 1b). They were still clearly seen even at 4W (Figs. 1c and d). On the other hand, in the control group, heparan sulphate proteoglycans were observed in small arterial walls and basement membranes of bile duct epithelial cells in the Glisson's capsule (Fig. 1e).

The number of Kupffer cells positive for ED2 was increased diffusely in the hepatic lobules at 2D when inflammatory changes were at their peak (Fig. 2a). After that, the number of Kupffer cells was decreased gradually, and it was almost the same with that in the control group at 4W (Figs. 2c and d). The size of Kupffer cells was larger in the GalN-group than in the control group (Figs. 2e and h). No spacial relationship was detected between Kupffer cells and proliferating oval cells (Figs. 2a-c). The cytoplasm of both proliferating oval cells and bile duct epithelial cells in the Glisson’s capsules showed clear stainability for TGF-β1 throughout the experimental period in the GalN-group (Figs. 3a-c). In the control group, clear positive stainability was also observed in the cytoplasm of bile duct epithelial cells in the Glisson’s capsules (Fig. 3e).

Discussion

In our previous study, almost all of the proliferating oval cells were positive for both α-fetoprotein, marker of fetal hepatocyte, and cytokeratin 7 (CK7), marker of bile duct epithelial cell, at 7D. However, almost all of the proliferating oval cells were positive only for CK7 at 4W. In addition, deposition of laminin and type IV collagen, which are the major components of basement membranes, was prominent around the proliferating oval cells throughout the experimental period. These results suggest that oval cells in this experimental model differentiate mainly into bile duct epithelial cells.

In this study, the deposition of heparan sulfate proteoglycans, one of the major components of basement mem-
Fig. 2. Immunostaining for Kupffer cells positive for ED2 in the liver of Mini rats. (a) At 2 D. Kupffer cells are diffusely increased in the hepatic lobules. (b) At 1 W. and (c) At 4 W. The number of Kupffer cells is decreased gradually. No special relationship is observed between Kupffer cells and the proliferating oval cells. (d) Control-group. (e) At 2 D. The size of Kupffer cells is larger compared with that in the control group. (f) At 1 W. and (g) At 4 W. The size of Kupffer cells is almost the same as that in the control group. (h) Control-group. Magnifications: (a) ×100, (b) ×100, (c) ×100, (d) ×100, (e) ×300, (f) ×300, (g) ×300, (h) ×300.
brane besides laminin and type IV collagen, was also clearly observed around the proliferating oval cells from 7D to 4W.

We also previously reported that activated Ito cells were observed in close relation with the proliferating oval cells even at 4W. On the other hand, in the present study, the spatial relationship between Kupffer cells and proliferating oval cells was not clear. This indicates that Kupffer cells may not play a direct role in the differentiation and/or proliferation of oval cells. However, it is reported that Kupffer cells are activated in the early stage of inflammation and then promote the activation of Ito cells.

There are several reports of immunostaining for TGF-β1 in the liver of rats. Among them, Lawrence et al. had reported the positive stainability for TGF-β1 in normal bile duct epithelial cells. Lawrence et al. said that mannos 6-phosphate/insulin-like growth factor II receptors (M6P/IGF IIR), which are located on the surface membrane of bile duct epithelial cells, are involved in the activation of latent-type TGF-β1. Latent-type TGF-β1 is generally activated by connecting to M6P/IGF IIR. However, some are not activated but internalized in bile duct epithelial cells. The internalized latent-type TGF-β1 is considered to show positive stainability in immunostaining. In this study, the cytoplasm of proliferating oval cells as well as bile duct epithelial cells showed positive stainability for TGF-β1. This may indirectly suggest the presence of M6P/IGF IIR on the cell surface of oval cells as well as bile duct epithelial cells. This may also indicate the differentiation of oval cells into bile duct epithelial cells.

It is difficult to determine immunohistochemically whether oval cells produce TGF-β1 or not, and therefore, we are now analyzing this point using the method of in situ hybridization. However, for the moment, we suppose that Ito cells may mainly produce TGF-β1 and that oval cells may modulate local concentration of the TGF-β1 by M6P/IGF IIR and then regulate the production of extracellular matrix by Ito cells. It is considered that the production of basement membrane components may participate in the induction of differentiation of oval cells into bile duct epithelial cells.

In conclusion, in this study, the accumulation of heparan sulfate proteoglycans was observed around the proliferating oval cells, and oval cells as well as bile duct epithelial cells showed positive stainability for TGF-β1. These results
also indicated the differentiation of oval cells into bile duct epithelial cells after the GaIN-administration. In addition, the present study suggested that Kupffer cells might be less important than Ito cells as a modulator of proliferation and/or differentiation of oval cells.

**References**


