A New Model for the Investigation of Pancreatic Hepatocytes
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In the previous examination of an acute infection of encephalomyocarditis (EMC) virus in Syrian hamsters of the APA strain, we have first succeeded in the induction of prominent hyperglycemia due to degranulation of pancreatic beta cells accompanying with marked pancratitis [13]. In the next experiment planned to clarify both the persistency of hyperglycemia and the development of pancreatic lesions in APA hamsters following EMC virus-infection, “pancreatic hepatocytes” were observed at a very high rate at 8 weeks after infection (8 WAI). Pancreatic hepatocytes were also found in EMC virus-infected Std: golden hamsters at a low rate at 8 WAI, but were not detected in mice at least up to 12 weeks after EMC virus-infection [1].

An induction of pancreatic hepatocytes has also been reported in some toxicity studies on some carcinogens in hamsters [8, 11] and rats [10]. Transdifferentiation of pancreatic cells into hepatocytes is an example of the surprising plasticity of cells in adult animals. However, the mechanism of transdifferentiation remains unknown for lack of suitable models for investigating pancreatic hepatocytes.

This paper describes the changes in blood glucose levels and pancreatic lesions as well as the incidence of pancreatic hepatocytes up to 8 WAI in EMC virus-infected APA hamsters.

A total of 55 one-month-old APA hamsters (43 males and 12 females) were used. Based on the results of the previous study [13], a suitable virus dose was chosen to induce prominent hyperglycemia at a very high frequency without animal loss. Namely, 43 animals (37 males and 6 females) were inoculated intraperitoneally with 10^3 PFU/ of the D variant of EMC virus (EMC-D; gifts from Dr. Ji-Won Yoon, The University of Calgary, Calgary, Alberta, Canada). Then, they were sacrificed by exsanguination under ether anesthesia at 1 (3 males), 2 (6 males), 4 (6 males), 6 (8 males) and 8 WAI (14 males and 6 females), respectively. The remaining 6 males and 6 females were killed at 8 WAI and served as histological controls. The animals were kept under controlled conditions (temperature, 24±1°C; relative humidity, 55±5%) in plastic cages with sterilized wood shaving for bedding. They were fed commercial pelleted diet, CMF (Oriental Yeast Co., Ltd., Tokyo) and tap water ad libitum.

Immediately after necropsy, the organs were fixed in 10% neutral buffered formalin, and 4 µm—paraffin sections were stained with hematoxylin and eosin (HE). For the detection of glycogen, some sections of the pancreas were treated with periodic acid-Schiff (PAS) or with Grifonia simplicifolia-II (GS-II) which reacts with terminal D-N-acetyl-glucosamine residues specifically. Moreover, other sections were also stained by avidin-biotin-peroxidase complex (ABC) method using Vectastain ABC Kit (Vector Laboratories Inc., Burlingame, CA, U.S.A.) for the detection of insulin granules in pancreatic islet beta cells. Anti-swine-insulin guinea pig serum (Scandibodies lab. Inc., Lakeside, CA, U.S.A.) was used as a first antibody.

Blood samples were obtained at 1-week intervals by orbital sinus blood collection from 12 (6 males and 6 females) of 20 animals which were finally killed at 8 WAI. Non-fasting glucose levels were colorimetrically determined on each serum sample using the Glucose C Test Kit.

Table 1. Changes in body weight and blood glucose level in APA hamsters infected with EMC-D

<table>
<thead>
<tr>
<th>Weeks after infection (WAI)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight(g)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Male</td>
<td>72.8 ± 6.2</td>
<td>66.9 ± 4.1</td>
<td>88.5 ± 4.3</td>
<td>107.1 ± 13.1</td>
<td>102.5 ± 6.9</td>
<td>115.9 ± 15.4</td>
</tr>
<tr>
<td>Female</td>
<td>70.7 ± 7.8</td>
<td>65.9 ± 4.1</td>
<td>88.8 ± 6.7</td>
<td>97.4 ± 5.9</td>
<td>113.7 ± 6.4</td>
<td>112.6 ± 2.9</td>
</tr>
<tr>
<td>Blood glucose level (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>125.4 ± 10.2</td>
<td>254.7 ± 123.1</td>
<td>187.6 ± 37.9</td>
<td>112.8 ± 22.4</td>
<td>131.1 ± 17.3</td>
<td>123.1 ± 40.3</td>
</tr>
<tr>
<td>Female</td>
<td>141.8 ± 31.2</td>
<td>294.4 ± 186.5</td>
<td>151.7 ± 114.5</td>
<td>111.6 ± 21.6</td>
<td>105.1 ± 4.7</td>
<td>115.0 ± 19.2</td>
</tr>
</tbody>
</table>

Data were taken from 6 animals infected with EMC-D and sacrificed at 8 WAI.
a) Mean ± S.D.
b) Significantly different from the values at 0 WAI (p<0.01).
(Wako Pure Chemical Industries Co., Ltd., Tokyo). Statistical analysis was performed using Student's t-test.

As reported in the previous paper [12], significant elevation of serum glucose levels was recorded at 1 WAI. The levels, however, began to lower at 2 WAI and returned to almost normal values at 4 WAI (Table 1). Thus, contrary to our expectation, it was clarified that hyperglycemia induced by EMC-D in APA hamsters was transient. We are now trying to induce persistent hyperglycemia in APA hamsters by an inoculation of EMC virus adapted to beta cell cultures prepared from APA hamsters.

In the pancreas, as reported in the previous paper [12], reduction in islet's size with degranulation and intracytoplasmic vacuolization of beta cells and replacement of necrotic tissue in the exocrine glands by mesenchymal cells and ductules were observed at 1 WAI. Thereafter, the islets became to show normal size with well granulated beta cells. On the other hand, in the exocrine glands, regeneration of acinar cells came to the fore in place of mesenchymal cells and ductules at 2 WAI. From 4 WAI on, regeneration of acinar cells progressed and the pancreas showed almost normal architecture at 6 WAI. Viral antigens were detected in both islet beta cells and acinar cells at 1 WAI as previously described [13].

In the organs other than pancreas, small focal myocardial fibrosis (heart), small focal neuronal loss (pyramidal layer of cerebrum) and atrophy of seminiferous tubules (testis) were observed at 8 WAI instead of previous parenchymal necrosis with inflammation at these sites in the acute phase [13].

Pancreatic hepatocytes were detected in 2 of 8 males at 6 WAI and in 11 of 14 males at 8 WAI (Table 2). Pancreatic hepatocytes were not found in females up to 8 WAI, and such sex difference in the incidence of pancreatic hepatocytes is a future problem to be solved. Non-infected control animals had no pancreatic hepatocytes.

Pancreatic hepatocytes were seen in a cluster and they located at the periphery of islets, in the midst of exocrine glands or in the adipose tissue (Fig. 1) with even distribution from the pancreatic head to the pancreatic tail. Pancreatic hepatocytes were polyhedral in shape and had centrally located nucleus and abundant eosinophilic cytoplasm (Fig. 1). Their cytoplasm was intensely PAS-positive (Fig. 2), becoming negative after predigestion with diastase, and was also GS-II-positive (Fig. 3), indicating the existence of glycogen granules. These histological and histochemical characteristics of pancreatic hepatocytes observed in the present study were similar to those reported previously as "pancreatic hepatocytes" or "hepatocyte-like cells in the pancreas" [3–5].

As mentioned before, pancreatic hepatocytes were also reported in hamsters and rats subjected to carcinogenic studies of such chemicals as N-nitrosobis (2-oxopropyl)amine [3, 7], 4-hydroxyaminoquinoline [12], azas Atene [5] etc. and to the combination study of ethionine administration with choline- or copper-deficient diet [2, 4, 5, 8, 9, 14]. However, some of these experimental systems require either complicated procedures or a long induction-period (more than 6 months), and others result in a low yield (less

<p>| Table 2. Incidence of pancreatic hepatocyte in APA hamsters infected with EMC-D |
|-----------------|---|---|---|---|---|</p>
<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8 WAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>0/3 (0%)</td>
<td>0/6 (0%)</td>
<td>0/6 (0%)</td>
<td>2/8 (25%)</td>
<td>11/14 (78.5%)</td>
</tr>
<tr>
<td>Female</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>0/6 (0%)</td>
</tr>
</tbody>
</table>

WAI: Weeks after infection of EMC-D.
NE: Not examined.
PANCREATIC HEPATOCYTE

Fig. 3. Pancreas of the same animal in Fig. 1. Pancreatic hepatocytes (arrowheads) are usually GS-II-positive. Lectin-staining, × 175.

than 40%) of pancreatic hepatocytes. Compared to them, the present experimental system could induce about a 80% yield of pancreatic hepatocytes in APA hamsters at 8 weeks after a single injection of EMC-D.

On the other hand, as to a hazard of EMC virus-transmission among rodents, we have recently clarified that virus excretion from infected animals ceased by 10 days after infection (DAI) and contact infection occurred only rarely even during this 10 days, of which details will be published elsewhere. This suggests that EMC-D-infected hamsters might have little possibility to cause horizontal transmission after 10 DAI.

From the above-mentioned findings, APA hamsters infected with EMC-D are considered to be a suitable model for the investigation of pancreatic hepatocytes. Detailed ultrastructural and histochemical investigations of pancreatic hepatocytes are now in progress focused on the period from 6 to 8 WAI in EMC-D-infected APA hamsters.

In conclusion, we could add a new model system suitable for investigating pancreatic hepatocytes.

REFERENCES