Brief Communication

Type C Viruses in the Pancreas of Normal C57BL Mice

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SUMMARY

Viral particles morphologically identical to type C viruses were found to be budding in large numbers from the membranes of intracytoplasmic vacuoles and of the endoplasmic reticulum of pancreatic acinar cells of untreated, normal C57BL mice more than 3 weeks old. In the thymus of the same animals and in the pancreas and thymus of newborn or 12-day-old mice, few particles were seen. No viral particles were observed in the pancreas of normal C3Hf and AKR mice.

INTRODUCTION

The thymus of the C57BL mouse is particularly sensitive to leukemogenesis by radiation or chemical carcinogens, and the induced tumors have been found to contain infectious virus (8, 10, 13). Yet no type C particles were observed in the thymus of embryonic and adult normal C57BL mice (12), although there was a preliminary report (2) of type C particles in embryonic thy muses of mice of the same strain. No evidence of type C viruses was found in C57BL mice until late in their lives by serological techniques that detected antibodies to the Gross leukemia antigen (1) or viral genome in the absence of infectious or visible particles (9).

In the course of a systematic search for viral particles in the thymus and other organs of mice subjected to a leukemogenic treatment with urethan (14), we came across an unexpected finding. This communication reports the intense multiplication of type C viruses in secretory cells of the exocrine pancreas of untreated C57BL mice.

MATERIALS AND METHODS

C57BL mice were obtained in 1961 from Dr. W. E. Heston; C3Hf mice were obtained in 1962 by cesarean section from a C3H/HeDp female and fostering on C57BL; AKR mice were obtained in 1969 from Dr. L. Chieco Bianchi. The 3 strains were bred thereafter by brother X sister mating in this laboratory. The number and ages of the animals examined are reported in Table 1.

The animals were killed with ether, and the pancreas and other organs of mice subjected to a leukemogenic treatment with urethan were excised, fixed at 4° for 2 hr in 3% distilled glutaraldehyde in Sörensen's phosphate buffer, and postfixed in 1% osmic acid in Millonig's phosphate buffer. Embedding was in an Epon-Araldite mixture. Ultrathin sections were placed on 200 mesh copper grids and stained with uranyl acetate, followed by Reynolds' lead citrate. From each specimen 3 blocks were cut, and 3 grids from each block were examined in a Hitachi HU-11 or Philips EM-300 electron microscope. A semiquantitative evaluation of the number of viral particles present in each animal was made by examining 3 squares in each grid.

RESULTS

Results are summarized in Table 1.

C57BL Mice. Typical type C viruses were seen in 29 of the 31 pancreases examined. In the 9 cases at 1 and 12 days of age, viral particles were rare, not more than 3 in the 9 grids scored for each animal, and these were always located in the extracellular spaces. The picture was strikingly different in the animals of 3 or more weeks of age. In all but 2 of the 22 cases examined, the acinar cells of the pancreas harbored a very large number of type C particles; most were located in large cytoplasmic vacuoles but some also were in the cisternae of the endoplasmic reticulum (Figs. 1 and 2). These vacuoles are not normally present and could be interpreted as dilated cisternae. Massive budding from the membranes of the vacuoles was frequently observed (Figs. 3 and 4), whereas only single particles were found to be budding from plasma membranes. Clusters of type C particles were also seen in the intercellular spaces. In contrast, 24 thymuses of the same animals (varying from 1 day to 108 weeks of age) revealed the presence of only a moderate number of type C particles, from 10 to 30 for each animal, uniformly distributed in the extracellular spaces and in vacuoles of epithelial cells.

C3Hf Mice. In none of the pancreases of the 9 C3Hf mice were type C particles observed, nor were dilated vacuoles seen. In the thymus of the same animals, the number and distribution of type C particles were essentially similar to that observed in C57BL mice.

AKR Mice. In the group of 5-week-old mice, viral particles were absent from the pancreas and were found in the thymus in only slightly greater quantity than in C57BL and C3Hf mice. The 4 mice of the 30- to 36-week-old group had an overt thymic lymphosarcoma. Two pancreases had no particles; while in the other 2, which were heavily infiltrated by leukemic cells, there was budding from the plasma membranes of acinar cells, and groups of type C particles were seen in the intercellular spaces. No vacuoles were observed. Numerous...
Table 1

Distribution of type C viruses in the pancreas and thymus of C57BL, C3Hf, and AKR mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>Age</th>
<th>No. of mice</th>
<th>No. positive/ no. tested</th>
<th>Comment</th>
<th>Pancreas</th>
<th>No. positive/ no. tested</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL</td>
<td>1 and 12 days</td>
<td>9</td>
<td>9/9</td>
<td>Rare, extracellular</td>
<td>9/9</td>
<td>Several, intra- and extracellular</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3–4 wk</td>
<td>8</td>
<td>7/8</td>
<td>Numerous, intra- and extracellular</td>
<td>8/8</td>
<td>Several, intra- and extracellular</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12–20 wk</td>
<td>10</td>
<td>9/10</td>
<td>Numerous, intra- and extracellular</td>
<td>10/10</td>
<td>Several, intra- and extracellular</td>
<td></td>
</tr>
<tr>
<td></td>
<td>108 wk</td>
<td>4</td>
<td>4/4</td>
<td>Numerous, intra- and extracellular</td>
<td>4/4</td>
<td>Several, intra- and extracellular</td>
<td></td>
</tr>
<tr>
<td>C3Hf</td>
<td>5 wk</td>
<td>3</td>
<td>0/3</td>
<td></td>
<td>3/3</td>
<td>Several, intra- and extracellular</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14 wk</td>
<td>3</td>
<td>0/3</td>
<td></td>
<td>3/3</td>
<td>Several, intra- and extracellular</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 wk</td>
<td>3</td>
<td>0/3</td>
<td></td>
<td>3/3</td>
<td>Several, intra- and extracellular</td>
<td></td>
</tr>
<tr>
<td>AKR</td>
<td>5 wk</td>
<td>4</td>
<td>0/4</td>
<td></td>
<td>4/4</td>
<td>Several, intra- and extracellular</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30–36 wk (leukemic)</td>
<td>4</td>
<td>2/4</td>
<td>Several, extracellular</td>
<td>4/4</td>
<td>Numerous, extracellular</td>
<td></td>
</tr>
</tbody>
</table>

type C viruses were found in the leukemic thymuses of the 4 animals.

DISCUSSION

At the present time, this finding might simply mean that cells with a high turnover of protein synthesis are able to support the maturation of large amounts of a virus, as already has been found for megakaryocytes (4). Feldman and Gross (6) reported the forming of type C particles in pancreatic acinar cells of leukemic adult C3Hf mice that, at birth, had received an injection of passage A virus.

The most important aspect of our observation, however, is related to the discovery of an abundant production of a virus, morphologically identical to the one known to produce leukemias and sarcomas in several species, in the pancreas of a murine strain that has a very low incidence of spontaneous leukemia but that is highly susceptible to leukemogenesis by radiation and chemical carcinogens. In this respect, our substrain seems to be no different from other C57BL substrains (5), and the lymphomas induced by chemicals have been demonstrated to contain antigens that cross-reacted with those present in Gross virus-induced lymphomas (3).

Surprisingly, no type C particles were found in the pancreases of normal C3Hf mice, which are susceptible to Gross virus leukemogenesis, or in those of normal AKR mice, which are overt lifelong carriers of Gross virus.

Kaplan (11) has clearly postulated that a release of virus must be the initiating step in radiation leukemogenesis, and Haran-Ghera (7) has provided experimental evidence of a leukemogenic activity of cell-free extracts of normal tissues of C57BL mice that 5 or 10 days previously had been given leukemogenic doses of radiation. Since the pancreas could very well be the source of the leukemogenic virus, we are now conducting in vivo and in vitro experiments to ascertain the biological activity of the virus produced by the pancreas. We are also planning to study the antigenicity of the virus in relation to a possible immunological control of its release.

ACKNOWLEDGMENTS

We are grateful to Dr. L. Lombardi for his advice and to Mr. Mario Azzini and Mr. Emilio Ferrara for technical assistance.

REFERENCES

7. Haran-Ghera, N. Leukemogenic Activity of Centrifugates from


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Fig. 1. Numerous mature, type C particles are present in a large cytoplasmic vacuole of an acinar cell of the pancreas of a normal adult C57BL mouse. X 38,000.

Fig. 2. Type C particles are budding at the membranes of the endoplasmic reticulum. X 106,000.

Figs. 3 and 4. Viral budding from the membrane of a cytoplasmic vacuole. X 60,000 and X 106,000, respectively.
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