HISTOLOGICAL SEXING OF A RETICULUM CELL SARCOMA ARISING AFTER LIVER TRANSPLANTATION


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Nuclear sex determination, based on both X and Y chromatin counts in a reticulum cell sarcoma which arose in the liver 5 months after orthotopic grafting, clearly showed that the tumor was of host origin. The Kupffer cells of the graft were also found to be of host origin, demonstrating that their replacement may occur within 6 months of operation.

There is an increased incidence of malignant tumors in transplant recipients receiving immunosuppressive drugs. For instance, the risk of developing a lymphoma is about 35 times higher than expected in the normal population, due largely to the incidence of reticulum cell sarcoma which is increased 350-fold. The tumor is usually assumed to have arisen de novo in the recipient, although in some cases, preexisting tumor, which had not been detected before operation, may have grown rapidly as a result of the immunosuppressive therapy. There has also been evidence in a few cases that tumor may have been transferred in the donor organ. In only one instance has the host origin of the malignant cells been demonstrated by histocompatibility serotyping of the tumor cells and both the donor and recipient lymphocytes. We describe a patient in whom a reticulum cell sarcoma confined to the allograft was found at necropsy approximately 5 months after orthotopic liver transplantation. The malignant cells were shown to be of host origin by histological sexing techniques.

Case Report

A 49-year-old woman had severe recurrent bouts of encephalopathy due to cirrhosis. The presence of Sjogren's disease and high titres of mitochondrial antibodies were suggestive of end-stage primary biliary cirrhosis. Some aspects of her clinical course have been reported previously (Case OL 224). Orthotopic liver transplantation was performed on October 10, 1970 by Professor R. Y. Calne; the donor organ was from a 17-year-old man with irreversible brain damage as a result of a traffic accident. Necropsy of the donor showed no evidence of tumor. Histocompatibility serotyping revealed antigen incompatibilities on HL-A 3, 11, and 7.

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The patient made a good postoperative recovery and was fully conscious by the 2nd day. Apart from a transient episode of jaundice at 2 months, thought to be due to rejection with superimposed cholangitis, she remained well until the 5th month when she again became jaundiced. A percutaneous transhepatic cholangiogram showed slight dilatation of the intrahepatic biliary radicles with narrowing, but not complete obstruction of the common hepatic duct. Her immunosuppressive therapy with prednisone and azathioprine was rapidly reduced in preparation for surgery, and intensive antibiotic therapy was instituted, but her general condition failed to improve and she died on March 30, 1971.

At necropsy, the biliary obstruction was found to be due to inspissated sludge and small calculi in the common hepatic duct without fibrous narrowing or external compression of the lumen. The cut surface of the liver showed discrete, greyish-white nodules, 1 to 2 cm diameter, scattered deep in the right lobe. The histological appearances were typical of reticulum cell sarcoma (fig. 1). There was no evidence of similar lesions elsewhere, and the spleen, which had been removed at the time of transplantation, showed only chronic congestive changes. Other necropsy findings included a large gastric ulcer, membranous enterocolitis, and Pneumocystis carinii pneumonia.

Materials and Methods

Liver tissue and tumor from the allograft was obtained a few hours after death. As control material, liver tissue from 28 consecutive necropsies, with or without liver disease, was obtained; the delay between death and necropsy ranged from 3 to 20 hr. Tissues were fixed in 10% formalin, were paraffin-embedded, and sections were cut at 5 to 7 μ.

The X-chromatin was studied using Feulgen-stained slides examined under oil immersion microscopy (x 800). For the study of the Y-chromatin, quinacrine fluorescence microscopy was used according to the method of Khudr and Benirschke with the following slight modifications. McIlvaine's buffer was used at pH 5.2 and the staining and rinsing times were varied for each case. The sections were examined with an oil immersion objective under a Zeiss fluorescence microscope (x 800).

The search for sex chromatin was performed on coded slides, without knowledge of the sex, by counting between 200 and 600 cell nuclei. The results are expressed as the mean number of positive nuclei per 100 cells counted. Hepatocytes and Kupffer cells were counted separately.
RESULTS

In the control livers, using the quinacrine fluorescence method, it was possible to identify the sex of each patient (15 males and 13 females) unequivocally, using either the hepatocytes or the Kupffer cells. The mean positive cell counts in the males were 46.6 and 38.9%, respectively, and for the females, 3.8 and 3.8%, with no overlap between the two (table 1.). With the Feulgen method on the liver cells (14 males and 10 females), such accurate sex differentiation is not possible, for although the mean values are significantly different in males and females \( (P < 0.001) \), there is some overlap (table 1.). However, using the Kupffer cells, there is clear separation of the sexes; the mean values for positive cells in males and females are 5.5% (range, 0 to 17) and 26.6% (range 24 to 59.5), respectively.

In sections of the liver graft (male donor), the values for X- and Y-chromatin counts in the hepatocytes fall within the range of our male controls (table 1.). In contrast, the counts in the tumor cells and Kupffer cells clearly point to a female origin for these cells, indicating that they were derived from the recipient (fig. 2).

DISCUSSION

The X-chromatin can be demonstrated in about 50 to 70% of nuclei from female organs, whereas male nuclei show an X-like body in less than 10% of their cells. However, the detection of the X-chromatin in the liver is difficult, for hepatocyte nuclei are often vesicular, and condensation of the chromatin into chromocenters gives rise to false positive counts. Cases with particularly large and clear nuclei may show no X-chromatin at all, leading to false negative results. Kupffer cell nuclei are more reliable, as the present results confirm.

Quinacrine staining of human chromosomes* and the discovery of the brightly fluorescent Y-chromosome visible also in interphase nuclei has led to another means of histological sex determination which is of particular value for liver tissue. On frozen sections, Kegel and Cohen found Y-chromatin in about 80% of male liver cells, while females showed 4.6% positive nuclei. Our lower mean count is presumably due to difficulty in obtaining specific fluorescence of the Y-chromosome in formalin-fixed and paraffin-embedded tissue. In the process of achieving optimal stain differentiation, some of the nuclei lose their specific staining and become very pale; however, such false negative results are easily recognized. The lack of fluorescence of the Y-chromosome in the tumor in our case was not due to technical reasons as the male liver cell nuclei present on the same section stained adequately. Loss of fluorescence of the Y-bodies as a consequence of chromosomal anomalies in malignant tissue is also unlikely to account for the negative findings, as other workers demonstrated Y-chromatin in most tumors arising in males.

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TABLE 1. Percentage of cells positive for X-chromatin by Feulgen method and Y-chromatin by quinacrine fluorescence in liver sections from patient and controls

<table>
<thead>
<tr>
<th>Staining method</th>
<th>No. of subjects</th>
<th>Hepatocytes</th>
<th>Kupffer cells</th>
<th>Tumor cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feulgen</td>
<td>Control males</td>
<td>14</td>
<td>4.9 (0-22)</td>
<td>5.5 (0-17)</td>
</tr>
<tr>
<td></td>
<td>Control females</td>
<td>10</td>
<td>29 (14-61)</td>
<td>36.6 (21-64)</td>
</tr>
<tr>
<td></td>
<td>Patient</td>
<td>1</td>
<td>2.5 (2-3)</td>
<td>30.5 (28-33)</td>
</tr>
<tr>
<td>Quinacrine</td>
<td>Control males</td>
<td>15</td>
<td>46.6 (29-71)</td>
<td>35.9 (20-52)</td>
</tr>
<tr>
<td></td>
<td>Control females</td>
<td>13</td>
<td>3.8 (2-7)</td>
<td>3.8 (1-7)</td>
</tr>
<tr>
<td></td>
<td>Patient</td>
<td>1</td>
<td>53.3 (43-63)</td>
<td>5.2 (4-7)</td>
</tr>
</tbody>
</table>

*Data are expressed as the mean percentage of positive cells. Numbers in parentheses indicate the range.
Furthermore, in our case, the percentage of tumor cell nuclei which were positive for the X-chromatin fell into the range of our female controls. The relatively low percentage of positive cells is in agreement with the findings of Moore and Barr\(^1\) that in malignant tissue arising in females, the mean number of positive nuclei is somewhat lower (54%, range 18 to 74%) than in benign tumors (74%) or in normal female tissue (72%). The fact that the tumor was host in origin, as demonstrated by histological sexing, and was confined to the transplanted organ, confirmed that it must have developed within the 6-month period from the time of transplantation. Penn and Starzl\(^2\) also reported that 8 of 35 mesenchymal tumors were discovered within 7 months of a transplant operation.

Another interesting finding in the present case is that the chromatin count in the Kupffer cells of nontumorous parts of the graft indicated a female origin for these cells. This is in agreement with studies of Porter\(^1\)\(^4\), using azure II staining for the X-chromatin on sections of transplanted liver, who found female Kupffer cells in 3 patients who had received a male liver 105, 380, and 380 days previously, whereas in 6 homografts studied ½ to 35 days after transplantation, the Kupffer cells remained male. Thus, after liver transplantation, the Kupffer cells of the donor are gradually replaced by cells of host origin and this repopulation may be achieved within 6 months of the graft.

REFERENCES


