Expression of Blood Group Antigen (A, B, H, Le\textsuperscript{a}, Le\textsuperscript{b}) on Liver Allografts

S. Hayashi, K. Noguchi, A. Yagihashi, T.E. Starzl, and Y. Iwaki

Recent studies clearly revealed that blood group-related carbohydrate determinants play a crucial role in cell-cell interactions and that blood group antigens such as A, B, H, Le\textsuperscript{a}, and Le\textsuperscript{b} were neoexpressed mainly on the bile duct or bile ductule in some liver diseases, although their detailed pathogenesis remained unclear.\textsuperscript{1,2} Thus, we examined the expression and the distribution of blood group antigens on liver allografts using immunohistochemical techniques.

MATERIALS AND METHODS

Patients

Twenty failed grafts were obtained from 20 patients who received orthotopic liver transplantation at the University of Pittsburgh Medical Center from December 1989 to June 1991. Seven grafts were lost because of primary nonfunction and the remaining 13 were due to rejections. The duration of graft failure ranged from 1 day to 2520 days. Their primary immunosuppressive therapy consisted of FK 506 or cyclosporine (CyA) in addition to a low maintenance dose of prednisolone. Ten liver tissues, on which biopsies were performed during hepatic resections due to other medical reasons, served as controls.

Immunohistochemical Examination

The following mouse anti-human monoclonal antibodies (MAbs) were used according to the blood group of the liver donor; anti-A, anti-B, and anti-H (DAKO Corporation, Carpenteria, Calif), anti-Lewis\textsuperscript{a} and anti-Lewis\textsuperscript{b} (Accurate Chemical and Scientific Co, Westbury, NY).

Immunoperoxidase staining was performed as followed: Failed liver allografts were frozen in an OCT compound and stored at -80°C until testing. Cryostat specimens of 5 \textmu m were initially incubated with avidin and biotin blocking reagents (Vector Laboratory, Burlingam, Calif) after fixation with acetone for 10 minutes. Then, the tissues were incubated with the anti-blood group MAbs for 30 minutes followed by the incubation with biotinylated second antibody (anti-mouse immunoglobulin) for an additional hour. After washing, these sections were reacted with avidin-biotin peroxidase complexes for further with 3,3'-diaminobenzidine substrate (Sigma, St Louis, Mo).

RESULTS

In the control, blood group antigens A, B, and H were expressed on the hepatic artery, portal vein, capillary, sinusoidal lining cells, and bile duct but not on the bile ductule. However, it was noted that the staining on sinusoidal lining cells was spotty. The expression of Le\textsuperscript{a} and Le\textsuperscript{b} in normal livers was restricted on the bile duct, bile ductule, and interlobular ductule.

An expression of A, B, and H antigen was markedly enhanced on capillaries (55%) and sinusoidal lining cells (65%) regardless of the cause of failure or the duration of failure (Table 1). There was an enhanced expression on hepatic artery and portal vein; however, it was not significant.

A significantly enhanced expression of Le\textsuperscript{a} and Le\textsuperscript{b} was also observed on the bile ductule in the majority of failed liver allografts (95%) (Table 2). In addition, 45% of failed graft had an enhanced Le\textsuperscript{a} and Le\textsuperscript{b} expression on the bile duct and 30% on the intralobular bile ductule.

DISCUSSION

This study clearly demonstrated that the expression of A, B, or H antigen was enhanced on capillaries or sinusoidal lining cells and that Le\textsuperscript{a} and Le\textsuperscript{b} antigens were also enhanced on capillaries (55%) and sinusoidal lining cells (65%) regardless of the cause of failure or the duration of failure (Table 1). There was an enhanced expression on hepatic artery and portal vein, however; it was not significant.

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Table 1. Enhanced Expression of A, B, and H Antigens in Failed Liver Allografts Compared to Those in Control

<table>
<thead>
<tr>
<th>Duration of Rejected Grafts (d)</th>
<th>0-3 (n = 7)</th>
<th>4-30 (n = 2)</th>
<th>31-90 (n = 6)</th>
<th>91&lt; (n = 5)</th>
<th>Total (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic artery</td>
<td>1*</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Portal vein</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>4 (20%)</td>
</tr>
<tr>
<td>Capillary</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>11 (55%)</td>
</tr>
<tr>
<td>Sinusoidal lining cells</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>13 (65%)</td>
</tr>
</tbody>
</table>

*Number of specimens that expressed enhanced expression compared to the control.

Table 2. Enhanced Expression of Le\textsuperscript{a} and Le\textsuperscript{b} in Failed Liver Allografts Compared to Those in Control

<table>
<thead>
<tr>
<th>Duration of Rejected Grafts (d)</th>
<th>0-3 (n = 7)</th>
<th>4-30 (n = 2)</th>
<th>31-90 (n = 6)</th>
<th>91&lt; (n = 5)</th>
<th>Total (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bile duct</td>
<td>1*</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>9 (45%)</td>
</tr>
<tr>
<td>Bile ductule</td>
<td>6</td>
<td>2</td>
<td>6</td>
<td>5</td>
<td>19 (95%)</td>
</tr>
<tr>
<td>Intralobular bile ductule</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>6 (30%)</td>
</tr>
</tbody>
</table>

*Number of specimens that expressed enhanced expression compared to the control.
significantly expressed primarily on the bile ductule. However, this enhanced expression did not seem to be associated with the duration or cause of liver failure. The carbohydrate blood group antigens particularly such as Le\(^a\) or Le\(^b\) were strongly expressed on proliferated ductules than those on normal ductules as reported earlier; however, the expression of A, B, H antigens was not dominant on the bile ductule. Thus, it might be probable that cell-cell interaction in the bile ductule system, when it is needed, could be communicated by primarily type 1 carbohydrate blood group antigens. It was interesting, however, that an enhanced expression on sinusoidal lining cells was primarily A, B, and H antigens, which belong to the type 2 carbohydrate blood antigen group.

It should be noted that A, B, and H antigens were also expressed in the failed grafts that were rejected within 3 days, all of which were due to primary nonfunction. Although there is a variety of pathologic features observed in the failed liver allografts including cholangitis, hepatitis, and acute or chronic rejections, primary nonfunctioning livers were partly a result of damage to the sinusoidal microvasculature. If this were the case, the enhanced expression of the blood group antigens on vascular beds would not only reflect the proliferation of those cells but would also reflect damaged tissues.

In summary, the expression of blood group carbohydrate antigen was enhanced in the failed liver allograft. The type 1 structure was dominantly expressed in the bile duct system and the type 2 expression seemed to be dominant on the capillary beds.

REFERENCES