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## The Lewis Blood Group System in Liver Transplantation

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**T**HE LEWIS blood group antigens Le<sup>a</sup> and Le<sup>b</sup> have been found on red blood cells (RBCs), in secretions and fluids such as saliva, alimentary tract juice, and urine, and in various tissues including renal tubular cells, collecting ducts, urothelium, and ductal or mucosal epithelium of the sweat glands, salivary glands, gastrointestinal tract, pancreas, uterus, cervix, and breast.<sup>1-8</sup> Hepatic bile duct epithelium also contains Lewis antigens.<sup>9</sup> Lewis antigens in secretions are glycoproteins. RBC Lewis antigens are glycolipids acquired from plasma and are not intrinsic to the RBCs.<sup>6,10</sup> Transfused RBCs have been found to assume the recipient's Lewis phenotype by absorption or loss of antigens within two to seven days,<sup>11,12</sup> and bone marrow transplant patients retain their own RBC Lewis phenotype, not that of their donors.<sup>13,14</sup> The site of origin of Lewis plasma glycolipids is uncertain. Evans et al<sup>15</sup> proposed the small intestine as a possible source. Crookston<sup>16</sup> suggested that the study of RBC Lewis phenotypes in liver transplants would shed light on this issue.

Lewis antibodies are generally clinically insignificant in RBC compatibility, in part because of the shift of transfused RBCs to the recipient's phenotype.<sup>12</sup> However, in renal transplantation, Lewis incompatibility has been reported to adversely affect graft survival.<sup>17-19</sup> Some Lewis antibodies are lymphocytotoxic<sup>19</sup> and others are not detected by routine hemagglutination techniques.<sup>18</sup> In one case an anti-host Lewis antibody of bone marrow graft origin was associated with renal failure in the recipient.<sup>20</sup> However, other clinical studies have not found a significant adverse effect of Lewis incompatibility in renal transplantation.<sup>21-23</sup>

On a random basis by prediction from Lewis phenotype frequencies,<sup>24</sup> an estimated 20% of whites and 35% of blacks getting

transplants from unrelated donors receive Lewis-incompatible organs. These figures are derived from the sum of approximately 5% of whites and 20% of blacks who are Le(a-b-) and predicted to receive Lewis-positive grafts, plus another 15% in each group who are Le(a+b-) and predicted to receive Le(a-b+) organs.

We studied Lewis-incompatible liver transplants with regard to the subsequent recipient RBC Lewis phenotype and the graft outcome. Our goals were twofold: (1) to assess the role of the Lewis blood group system in hepatic transplant compatibility, and (2) to determine whether the liver is the source of RBC Lewis antigens.

### MATERIALS AND METHODS

Patients were studied in the liver transplant program at the University of Pittsburgh. Surgical and immunosuppressive methods and transfusion practices have been described.<sup>25-28</sup> Children under 6 years of age were excluded in our study because young children do not fully express RBC Lewis antigens.<sup>12</sup> Patients with mixed pretransplant RBC Lewis typings attributed to recent transfusions were excluded from our prospective cases. RBC Lewis typings were performed in standard fashion on clotted or ethylenediaminetetraacetate (EDTA)-anticoagulated specimens from the patient prior to transplant and from the organ donor when available. Posttransplant Lewis typings were done at least 11 days after the most recent RBC transfusion, except in one case (see Results). Some posttransplant typings were performed on heparinized specimens sent for cyclosporine levels and kindly provided by Howard Seltman. Patient 2's posttransplant Lewis typing was kindly arranged by Arthur S. Lebowitz and the New York University Medical Center blood bank. We performed the secretor study with saliva using

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hemagglutination inhibition.<sup>24</sup> Patient followup was included through January 1, 1987.

### RESULTS

Seventeen definite or probable Lewis-incompatible liver transplants were studied in 12 patients (Table 1). Patients 1 through 3 were identified retrospectively because of having had Lewis antibodies; the others were found in a prospective search for Le(a-b-) or Le(a+b-) recipients. Ten Le(a-b-) patients received 15 grafts. RBC Lewis typings were done on four of these organ donors; the other 11 were most likely Lewis incompatible because only 6% of whites and 22% of blacks are Le(a-b-).<sup>24</sup> Two Le(a+b-) patients (cases 7 and 9) received Le(a-b+) livers.

Excluding graft 11C, for which only 1 month's follow-up has elapsed, 9 of the 16 grafts (56%) in Table 1 were surviving at 7 to 44 months. This rate is comparable to our center's overall 6-month actuarial survival of 58% for all hepatic grafts.<sup>29</sup> Two patients were retransplanted after rejection (graft 6A, which also had arterial thrombosis present,

and grafts 11A and 11B); their current grafts were surviving at 13 months and 1 month. Ten of these 12 patients (84%) were surviving  $\geq 7$  months after transplant, compared to our program's overall 6-month actuarial patient survival rate of 73%.<sup>29</sup> The two deaths were not related to rejection. Thus, the presence of Lewis incompatibility did not adversely affect overall patient or graft survival.

In 13 of the 17 transplants, RBC Lewis typing was performed after the transplant. The interval from transplant to typing is shown in Table 1. Patient 1 is presumed to be originally Le(a-b-) because of the presence of anti-Le<sup>bH</sup> before graft B (see below). All posttransplant typings shown were done  $\geq 11$  days after the most recent RBC transfusion, except in case 12B, in which repeated transfusions were needed and the Le(a-b-) typing was obtained 6 days after 2 units of RBCs. All retained their original Lewis RBC phenotype except for patient 4, whose RBCs were Le(a-b+) 3 weeks and 8 months posttransplant; her saliva also contained Le<sup>b</sup> in a secretor study at 3 weeks. Postoperative RBC specimens were not obtained after transplants

Table 1. RBC Lewis Phenotypes and Outcomes of 17 Definite or Probable Lewis-Incompatible Liver Transplants

| Patient-Graft | Disease    | Donor              | RBC Phenotype      |                                  | Graft Survival                            |
|---------------|------------|--------------------|--------------------|----------------------------------|---|
|               |            |                    | Preop              | Postop                           |   |
| 1A            | CAH        | NT                 | NT                 | Le <sup>a-b-</sup> , 11 d        | 11 d, arterial thrombosis                 |
| 1B            |            | NT                 | Le <sup>a-b-</sup> | NT                               | 44 mo+                                    |
| 2             | NANBH      | NT                 | Le <sup>a-b-</sup> | Le <sup>a-b-</sup> , 22 mo       | 41 mo+                                    |
| 3             | Alagille's | NT                 | Le <sup>a-b-</sup> | Le <sup>a-b-</sup> , 16 d        | 20 mo+                                    |
| 4             | Wilson's   | NT                 | Le <sup>a-b-</sup> | Le <sup>a-b+</sup> , 21 d + 8 mo | 16 mo+                                    |
| 5             | CAH        | NT                 | Le <sup>a-b-</sup> | Le <sup>a-b-</sup> , 7 mo        | 14 mo+                                    |
| 6A            | CAH        | NT                 | Le <sup>a-b-</sup> | Le <sup>a-b-</sup> , 16 d        | 1 mo, rejection + arterial thrombosis     |
| 6B            |            | NT                 | Le <sup>a-b-</sup> | Le <sup>a-b-</sup> , 6 mo        | 13 mo+                                    |
| 7             | CAH        | Le <sup>a-b+</sup> | Le <sup>a+b-</sup> | NT                               | 13 mo+                                    |
| 8             | CAH        | Le <sup>a-b+</sup> | Le <sup>a-b-</sup> | Le <sup>a-b-</sup> , 1 mo        | 10 mo+                                    |
| 9             | CIR        | Le <sup>a-b+</sup> | Le <sup>a+b-</sup> | Le <sup>a+b-</sup> , 1 mo        | 7 mo+                                     |
| 10            | PBC        | NT                 | Le <sup>a-b-</sup> | Le <sup>a-b-</sup> , 15 d        | 5 mo, died, <i>Pneumocystis pneumonia</i> |
| 11A           | PBC        | Le <sup>a-b+</sup> | Le <sup>a-b-</sup> | Le <sup>a-b-</sup> , 7 wk        | 4 mo, chronic rejection                   |
| 11B           |            | NT                 | Le <sup>a-b-</sup> | NT                               | 4 mo, acute rejection                     |
| 11C           |            | NT                 | NT                 | Le <sup>a-b-</sup> , 25 d        | 1 mo+                                     |
| 12A           | CHF        | Le <sup>a-b+</sup> | Le <sup>a-b-</sup> | NT                               | 1 wk, harvest ischemia                    |
| 12B           |            | Le <sup>a+b-</sup> | NT                 | Le <sup>a-b-</sup> , 23 d        | 1 mo, died, stroke                        |

Abbreviations: CAH, chronic active hepatitis; NT, not tested; NANBH, non-A, non-B hepatitis; CIR, cirrhosis; PBC, primary biliary cirrhosis; CHF, congenital hepatic fibrosis.

1B, 7, and 11B, and were not done after case 12A because of retransplantation 1 week after the transfusions with graft 12A.

The postoperative typings in Table 1 were the initial ones performed, except in patients 5 and 8 where transfused Le(b+) RBCs were still detected at 17 days in each case. This interval was somewhat longer than in other reports.<sup>11,12</sup> These two patients received 13 and 18 units of RBCs at transplant, respectively. The other six patients who were retyped from 11 days to 1 month after transplant, and who retained their original Lewis phenotype, received from 3 to 11 units of RBCs (cases 3, 6A, 8, 9, 11C, and 12B). All of these patients received intraoperative fresh frozen plasma (FFP) on a one-for-one basis with RBCs,<sup>27,28</sup> and plasma transfusion is also a source of Lewis antigens.<sup>12</sup> Thus, transfusions of up to 11 units of RBCs and FFP at the time of transplant did not affect results of postoperative RBC Lewis phenotyping 11 days to 1 month later.

Ten control patients receiving Lewis-compatible livers also had postoperative RBC Lewis phenotypings. Nine were Le(a-b+) patients whose donors were Le(a-b+) (3), Le(a+b-) (2), or unknown (3), and one was Le(a+b-) with an Le(a+b-) liver. Le(a-b+) subjects are compatible with Le(a+b-) blood or tissue, and do not make anti-Le<sup>a</sup>, because Le<sup>a</sup> is the precursor for Le<sup>b</sup> and Le(a-b+) subjects have small amounts of circulating Le<sup>a</sup>. All of these ten patients retained their original Lewis phenotype.

Three patients had pretransplant serum Lewis antibodies. Anti-Le<sup>bH</sup>, which reacts most strongly with group O and A<sub>2</sub> Le(b+) RBCs having abundant H substance, was detected prior to grafts 1B and 2. Patient 2 was group B and received a group O liver (high H content). She subsequently developed anti-B from graft lymphocytes and in that context was reported previously by Ramsey et al.<sup>30</sup> (patient 2 of that series). Patient 3 had preoperative serum anti-Le<sup>a</sup> and anti-Le<sup>b</sup> reacting weakly to enzyme-treated RBCs in

indirect antiglobulin testing. At 16 days after surgery, the strength of the anti-Le<sup>b</sup> had increased to 3+ while the anti-Le<sup>a</sup> was unchanged. Despite these preoperative Lewis antibodies, all three of these grafts are surviving.

#### DISCUSSION

In our study, Lewis incompatibility did not result in increased graft loss. Six-month graft survival in our 17 definite or probable Lewis-incompatible grafts was 56%, compared to 58% in our program's overall experience.<sup>29</sup> Liver transplants are also relatively more tolerant of ABO and HLA mismatching than renal grafts. Although ABO compatibility is sought whenever possible, a number of successful ABO-incompatible liver transplants have been performed in our program.<sup>29</sup> Also, hepatic grafts are performed without regard for HLA matching or lymphocytotoxicity crossmatching.<sup>31</sup> In contrast, renal transplants frequently undergo hyperacute rejection in ABO incompatibility, are adversely affected by HLA mismatching and incompatibility, and in some series have had reduced survival when Lewis incompatibility is present. Our findings with the Lewis blood group system in liver transplantation are consistent with the generally greater tolerance of the transplanted liver for other tissue incompatibilities.

We have also shown that the liver is generally not the source of RBC Lewis glycolipids. In 12 of 13 definite or probable Lewis-incompatible liver transplants, the recipient retained his or her original RBC Lewis phenotype. All recipients were Le(a-b-) except for two Le(a+b-) patients who received Le(a-b+) livers. The single apparent RBC phenotype switch was in a patient with Wilson's disease who appeared to be Le(a-b-) preoperatively, but Le(a-b+) postoperatively. In retrospect in this patient, we cannot rule out the possibility of preoperative weakened RBC Lewis expression, which has been observed in the settings of pregnancy, alcoholic cirrhosis

and pancreatitis, and chronic renal failure.<sup>19,32,33</sup> In contrast to alcoholic cirrhosis, Stigendal et al<sup>33</sup> found normal expression of RBC Lewis antigens in chronic active hepatitis and primary biliary cirrhosis. Further studies would be required to determine whether Wilson's disease might be associated with reduced RBC Lewis expression in some patients.

Evans et al<sup>15</sup> proposed that Lewis antigens in the small intestinal lumen are processed by the mucosa into small dialyzable forms that are then absorbed into the plasma and excreted in the urine. However, the material

they studied in the small intestine was presumed to be glycoprotein, not glycolipid as is present on RBCs. Our data appear to rule out enterohepatic-plasma circulation of RBC Lewis antigens. Further information on possible small intestinal origin of plasma Lewis glycolipids could be gleaned from future studies of small intestinal transplantation.<sup>34</sup> We have studied an Le(a-b-) patient who received a pancreas transplant (unknown donor Lewis type), including the spleen and a small cuff of duodenum; the patient's RBCs were still Le(a-b-) at 7 weeks, and the graft is functioning at 15 months.

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