



## Proteome Analysis in Liver Transplantation

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**T**HE USE of the DNA database does not allow for an understanding of how a cell or whole tissue functions at the molecular level before or after transplantation. Therefore, attention has increasingly turned toward the identification and characterization of the functional protein products encoded by the genome of an organism or tissue, and thus the process of proteomics has been coined to describe this approach. In the present study, proteome analysis is applied to liver transplant research.

### MATERIALS AND METHODS

Orthotopic liver transplantation (OLT) was performed under anesthesia using Kamada's method with some modifications.<sup>1</sup> Rat serum was taken at various timepoints from tolerogenic orthotopic liver transplantation (OLT) (DA-PVG) and syngeneic OLT (DA-DA) models. All serum samples were immediately aliquoted and frozen at  $-70^{\circ}\text{C}$ .

#### First Dimension Gel

Isoelectric focusing (IEF) and SDS-PAGE<sup>2</sup> were performed. Electrophoresis was done using the Pharmacia IPGphor apparatus according to the manufacturer's instructions, with slight modification. Following IEF, the gel strips were loaded with SDS in two 15-minute equilibration stages. During these steps, the Protean II apparatus was prepared for vertical SDS-PAGE. Electrophoresis was performed at constant current; that is, 24 mA/gel until the tracking dye reached the cathode. Immediately after SDS-PAGE the gels were immersed in a fixing solution of 40% v/v ethanol and 10% v/v acetic acid. The Pharmacia PlusOne staining kit was used.

### RESULTS AND DISCUSSION

#### Protein Stain

The patterns of protein expression were initially analyzed in the serum and liver tissue before and after OLT (DA donor into PVG). Despite a fully allogeneic barrier between the DA donor and PVG recipient, rejection was naturally overcome, followed by induction of tolerance. On day 60, when tolerance was established in this OLT model, nine proteins in the serum were differentiated from the untransplanted liver and serum by two-dimensional SDS-PAGE.

Alternatively, naive PVG serum showed 13 differentiated spots compared with the OLT serum. These proteins were further identified by a combination of microsequencing, in-gel digestion, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF), and database searches coupled with peptide-mass fingerprinting.<sup>3,4</sup> In the OLT serum, two of a total of five two-dimensional-differentiated proteins showed high homology with APO-E and clusterin (a complement activation regulatory factor). APO-E also has been reported to suppress T cells in vitro. However, 1 of the 13 spots found in the PVG serum was a vitamin D-binding protein, which was not seen in the OLT serum. This may explain why OLT patients often suffer from bone fracture or osteoporosis as a posttransplant phenomenon. The application of proteome analysis to liver allograft tolerance promises to provide valuable therapeutic and diagnostic strategies for induction of drug-free tolerance in the transplant setting.

### REFERENCES

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