Expression of Clusterin in a Rat Tolerogenic OLT Model


In rat orthotopic liver transplantation (OLT), PVG recipients of DA livers (tolerogenic model) survive without immunosuppression and become tolerant of subsequent grafts of other DA organs such as skin, heart, and kidney.1,2 Many aspects of the mechanism regarding this naturally achieved tolerance have been discussed in our previous reports. However, the mechanism of liver allograft tolerance from the aspect of the complement system and its regulatory proteins has not yet been investigated. The complement system comprises a group of proteins that interact with other immune system molecules to provide many of the effector functions of humoral immunity and inflammation.3 Clusterin, which is a plasma glycoprotein, regulates the complement system by inhibiting the membrane attack complex (MAC) formation.3 Here, we studied the kinetics of clusterin expression after transplantation in the tolerogenic model (DA-PVG) compared with syngenic model (DA-DA) and acute rejector model (DA-LEW) by immunoblotting and Northern blotting.

MATERIALS AND METHODS

Animals and Orthotopic Liver Transplantation (OLT)

Male inbred DA (RT1a), PVG (RT1c), and LEW (RT11) rats were used. OLT was carried out in the combination of DA to PVG, DA to LEW, and DA to DA by a previously described technique.1

Immunoblotting

Sera were taken from OLT rats at postoperative days (POD) 7, 14, and 60 and then stored at −70°C until used. Sera (~30 μg) and molecular weight standards (BioRad) were run on SDS-PAGE using 10% precasted gels (BioRad). After electrophoretic separation, proteins were transferred to 0.45-μm nitrocellulose membrane (BioRad) using a Semi-Dry Transfer Cell unit (BioRad). The nitrocellulose membranes were blocked by incubation in 5% (w/v) nonfat milk in Tris-buffered saline containing 0.05% Tween-20. Rabbit anti-rat clusterin polyclonal IgG (Upstate, USA) and goat anti-rabbit immunoglobulin alkaline phosphatase conjugate substrate (Sigma) were used as primary and secondary antibodies. The membranes were developed in BCIP/NBT alkaline phosphatase substrate (Sigma) until bands appeared.

Northern Blotting

Total RNA was extracted from liver tissue taken on POD 7 from OLT rats with Tri Reagent (Molecular Research Center Inc) according to the manufacturer’s instructions. Specific probes were generated by labeling re amplified or cloned c-DNA fragments with digoxigenin-11-dUTP (Random Primer DNA labeling kit, Boehringer Mannheim). Clusterin mRNA were detected by a colorimetric method with NBT/BCIP (Boehringer Mannheim). β-Actin was used as an internal control for northern blotting.

RESULTS

Immunoblotting

The levels of clusterin were measured and calculated as a ratio of optical density of POD samples divided by control (naïve PVG). Strongest expression of clusterin was observed on POD 7 (mean = 5.04 ± 0.39) (statistic software SPSS), when rejection occurred in the tolerogenic model, and gradually decreased (mean = 3.69 ± 0.25 ~ 1.78 ± 0.15) afterward. A significant difference in clusterin expression was observed between the tolerogenic model and syngenic model (5.04 ± 0.39 vs 3.54 ± 0.24) on POD 7 and POD 60 (1.78 ± 0.15 vs 0.27 ± 0.08).

Northern Blotting

Clusterin mRNA levels were measured and calculated in a ratio of optical density of samples divided by an internal control (β-actin). Strongest expression of clusterin was observed in the tolerogenic model (1.07 ± 0.09) on POD 7 compared with the syngenic model (0.63 ± 0.03) and an acute rejector model (0.60 ± 0.01).

DISCUSSION

The liver is a major source of complement components, but extrahepatic sources of complement have been hypothesized to contribute to inflammation and hyperacute rejection.4 Clusterin is the inhibitory protein of MAC that is generated by complement activation, suggesting that clusterin may serve as an antiinflammatory agent during rejection.

From the Department of Surgery and Liver Transplant Program, Chang Gung Memorial Hospital, Kaohsiung, Taiwan (K.C.C., C.L.C., C.L.L., Y.C.L., T.L.P., C.Y.L., H.P.T., L.W.H., H.Y.H., S.G.); Department of Cardiology, The University of Wales College of Medicine, Cardiff, United Kingdom (R.L.); and Department of Surgery I, Oita Medical University, Oita, Japan (H.Y., S.K.).

Address reprint requests to Shigeru Goto, MD, 123 Ta-Pei Rd, Niao Sung, Kaohsiung, Taiwan.

© 2000 by Elsevier Science Inc.
655 Avenue of the Americas, New York, NY 10010

tion. The protein is thought to be involved in maintaining membrane integrity or membrane remodeling, and in many cases clusterin expression is up-regulated in instances of cell injury.\textsuperscript{5} This may explain the continuing presence of clusterin detected in the tolerogenic model after the tolerance is achieved.

Clusterin is a widely distributed and highly conserved protein for which many functions have been proposed. Previous reports have shown that high levels of clusterin in transfected L929 cells potentiated the cytotoxicity of TGF-\(\beta\) and that exposure of L929 cells to TGF-\(\beta\) provided protection against TNF-\(\alpha\).\textsuperscript{6} TGF has been reported to be up-regulated during rejection. Clusterin has been identified as binding with the intracellular domains of TGF receptors type I and II, thereby conferring resistance to cells to TNF-\(\alpha\).\textsuperscript{7} We speculate that increased levels of clusterin detected during rejection is related to the up-regulation of TGF, suggesting that clusterin and TGF-\(\beta\) act via a common mechanism to provide protection against the cytotoxicity of TNF-\(\alpha\) and contribute to the induction of tolerance.\textsuperscript{6} Further studies on TGF-\(\beta\) and TNF-\(\alpha\) in relation to the induced tolerance will be investigated. We expect to observe an increase in the level of clusterin along with an upregulation of TGF-\(\beta\), related to a decreased level of TNF-\(\alpha\).

In conclusion, expression of clusterin may serve as a tolerance marker, and studies of TGF-\(\beta\) and TNF-\(\alpha\) signal pathways and complement proteins are involved in the induced tolerance and need further analysis.

ACKNOWLEDGMENT

This work is supported by National Science Council (NSC89-2314-B-182A-062), Taiwan.

REFERENCES